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GROWTH AND FLOWERING OF SOME TAME AND WILD GRASSES IN RESPONSE TO DIFFERENT PHOTOPERIODS[1]

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INTRODUCTION

In 1934 a paper appeared under the joint authorship of Evans and Allard (*7*)[2] embodying the results of a study of the growth and flowering behavior of 16 strains of timothy in response to different constant daily light periods, all but 4 strains being of American origin. The 4 not of American origin originated from seed obtained from northern Europe and were shown to attain the flowering condition at later dates than the American strains. It was indicated in this paper that the observed earliness or lateness of the various timothy strains was mainly due to inherent differences of response to the seasonal length of day following the awakening of growth in springtime.

Further discussions of the length-of-day behavior of timothy strains were embodied in papers by Evans, Allard, and McConkey in 1935 (*8*), and by Evans in 1939 (*6*).

Earliness of flowering appears to depend upon the fact that some varieties lay down the flower primordia in response to shorter day lengths than the later flowering varieties. The latter must await the coming of the longer days of summertime before the flower primordia, if initiated, can develop into a normal flower.

Varieties have been found that in the latitude of Washington, D. C., flowered very sparsely or not at all, even when the length of the midsummer day had attained its greatest value, nearly 14.9 hours. The English strain of timothy known as Harpenden, reported upon in the present paper, is of this type.

In view of the fact that this study of some of the more important tame grasses, begun by Evans and Allard, has been extended to include a number of other very important lawn and forage types, it has seemed advisable to present the behavior of these in the present paper.

It is well known that vast areas of the United States in the West are comprised of natural grasslands. It is also obvious that, in the great forest belts of the more humid regions, the woodland on the more arable soils everywhere has had to give way to an artificially established grassland, which comprises lawns and pastures and also meadows for the production of crops of hay in the dairy and stock-raising regions. In other words, the American system of agriculture to a great degree involves the cultivation of the various grasses for one purpose or another, and much of the success in grassland agriculture

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[2] Italic numbers in parentheses refer to Literature Cited, p. 227.

has come with the successful introduction of European types adapted originally to European fields and climate.

In the past the introduction of many exotic grasses has been largely an empirical procedure. With the discovery of the importance of the principle of the length-of-day responses of plants in 1920 by Garner and Allard (*9*), botanists have come to realize that the successful introduction of useful plants into new regions may depend largely upon their length-of-day behavior, as well as upon their temperature limitations and other factors. Obviously far northern plants are adapted to the long days and cool temperatures of high latitudes, and their flowering and fruiting, other conditions being favorable, can be expected in approximately similar temperatures and latitudes both north and south of the equator. Whether or not such plants can be grown under the shorter equatorial days depends as much upon the range of their temperature and day-length responses as upon any other factor. As a matter of fact, if high temperature is not unfavorable, length of day may often be the limiting factor, since temperatures low enough to interfere with growth do not ordinarily occur in equatorial regions.

In many plants, especially the herbaceous grasses, the character of the vegetative growth, as well as that of flowering and fruiting, may be profoundly altered by the length-of-day factor. Under one set of conditions the plants may produce flowering stems abundantly, with the expression of sexual reproduction dominant. Under another set of day-length conditions, sexual reproduction may be entirely or partially suppressed and vegetative growth accentuated. The dominance of this phase of growth expression leads to vigorous sod formation by stooling, stolon formation, and nodal branching.

These two categories of growth behavior to a certain extent may be antagonistic, since factors causing a strong dominance of one may retard or weaken the expression of the other. Since they represent continuities of a single life history, however, it is evident that certain borderline lengths of day may be favorable to a close interplay of both activities. In such instances, purely vegetative growth is more nearly in equilibrium with sexual reproductive expression. It is here that the largest growth form and the most vigorous fruiting may take place, favorable to high hay and forage yields.

In the case of the lawn grasses a greater degree of vegetative dominance is more naturally expected, for this leads to better mat or sod formation owing to a rapid lateral extension of the original plant, with little or none of its energies given over to flower-stem and seed formation.

A basic and thorough understanding of the ecological effects of seasonal length of day alone upon the life history of the grasses involves considerable study and analysis. The seasonal length of day in the middle latitudes is ever an inconstant condition. Increasing amplitudes of light prevail from spring to midsummer, and decreasing values from midsummer to autumn. The former condition favors sexual expression in the long-day grasses; the latter, in the short-day grasses. As has been indicated above, when sexual expression is attaining its maximum condition purely vegetative expression is suffering suppression, and the converse relation obtains when vegetative expression is at its maximum. This seasonal interplay of wide-

limit variations in length of day exercises very marked effects upon the development of vegetation in the higher temperate latitudes and very largely determines the life history of the grasses as we see their expression in American farm economics. In equatorial regions the seasonal swing becomes very narrow, and if there are day-length responses in the vegetation these must depend upon very small increments and decrements. It is evident that in the high Arctic latitudes, where constant daylight obtains throughout the growing season, the plants experience no seasonal changes in length of day, and such phases of development as they show must depend upon other factors than light-darkness ratios. A slight change in latitude near the critical length of day for the best expression of a particular phase of growth may thus profoundly modify the entire course of development of the particular species and militate against its usefulness for a given purpose. It must be understood, however, that length of day is but one of many factors operating at all latitudes, and that temperature, more especially, may greatly modify the developmental processes.

In the propagation of the grasses vegetatively, likewise, there may be decided advantages depending upon the suitable length-of-day factor. It would appear that, when vegetative dominance is at its maximum, rapid growth and extension of the clones is most favored. For this reason vegetative propagation is favored under those lengths of day that are less than the critical length for flowering or sexual reproduction. Vegetative propagation of the long-day grasses in the greenhouse in wintertime is dependent upon this principle, and the rapid lateral extension of the clumps under similar short photoperiods artificially produced in summertime is additional proof of its operation.

A proper and conservative recognition of the length-of-day factor, therefore, as affecting the expression of the grasses, may not only clarify the logic of much of the empirical success in the past, but make it possible to handle and to distribute newer introductions in a more scientific manner and therefore with greater success.

MATERIAL AND METHODS

The investigations reported upon were carried out at Arlington, Va., with the equipment of the Division of Tobacco Investigations located there.

The present paper deals with 13 species and varieties or strains of grasses, including the following:

Canada bluegrass (*Poa compressa* L.), introduced from the Old World (table 2).
Kentucky bluegrass (*Poa pratensis* L.), introduced from the Old World (table 3).
Orchard grass (*Dactylis glomerata* L.), introduced from the Old World (table 4).
Wirestem muhly (*Muhlenbergia mexicana* (L.) Trin.), native of North America (table 5).
Nimblewill (*Muhlenbergia schreberi* Gmel.), native of North America (table 6).
Reed canary grass (*Phalaris arundinacea* L.), introduced from the Old World (table 7).
Smooth bromegrass (*Bromus inermis* Leyss.), introduced from the Old World (table 8).
Timothy (*Phleum pratense* L.), introduced from the Old World (tables 9 and 10).
Bulbous bluegrass (*Poa bulbosa* L.), introduced from Europe (table 12).
Creeping bent (*Agrostis palustris* Huds.), native of America and Europe (table 13).
Indian grass (*Sorghastrum nutans* (L.) Nash), native of North America (table 14).
Bottlebrush (*Hystrix patula* Moench), native of North America (table 15).
Eastern gamagrass (*Tripsacum dactyloides* (L.) L.), native of North America (table 16).

Most of these grasses are among our most valuable species, having a high degree of usefulness for one purpose or another in American grassland agriculture.

In the present paper, three strains of Kentucky bluegrass are being reported upon, one early, one medium-late, and one a late strain. Five strains of timothy have been tested, including one of Harpenden, England, origin; one a Russian (Moscow) strain; one designated as Welsh S. 50, a pasture variety; and the American varieties Huron (3937) and Marietta (11901).

Two strains of creeping bent (*Agrostis palustris*) that have come into use for lawns and golf greens have been studied. These are known as Metropolitan bent and Washington bent.

The majority of the grasses reported upon in this paper are Old World natives that have long been established in North America. Many of these species have a very wide distribution, and the results of the present study would indicate the importance of day-length studies for grasses in general.

Indian grass (*Sorghastrum*) and bottlebrush (*Hystrix*) are familiar wild North American species, the former being a common constituent of the tall-grass prairies of the Middle West and a normal grass component of the old-field successions of the East. Bottlebrush is confined almost strictly to deciduous woodlands. It is a beautiful and graceful grass, but of no present economic value. Oftentimes it occurs in patches of considerable size in the heavy woodlands of the mountain areas; and on some of the shale barrens of the Massanutten ridges in Virginia it appears to have a place in certain successions, forming a dense ground cover where duff and humus have accumulated.

The three timothy strains, Harpenden, Russian (Moscow), and Welsh strain S. 50, were received as large clumps from Canada, through the efforts of Prof. O. McConkey, of Agricultural College, Guelph, Ontario. These clumps were divided March 18, 1936, potted in 3-inch pots, and kept in a cool greenhouse with temperatures ranging from 50° to 55° F. On March 28, 1936, these pots were transferred to open coldframes to keep the plants dormant so far as possible. These plants made excellent growth, filling the small pots with leafage, and on March 30 they were transferred to 14-quart galvanized-iron buckets and to tests when about 2 inches in height.

The two timothy varieties, F. C. 3937 and F. C. 11901, were received as large clumps from Ohio, February 1937. The former was divided and potted in thumb pots February 23 and kept in the cool house until April 7, when the plants were transferred to pails and tests, the leafage then being 3 inches tall.

The clump of 11901, after being divided, was planted in 4-inch pots February 10 and kept in the cool house (50° to 55° F.) until March 4, when the plants were transferred to the coldframe out of doors. This material was finally placed in pails and transferred to the tests March 31, when the leafage was 6 inches tall.

All the remaining species, except the creeping bents, the Indian grass, and the bottlebrush, were received as large clumps from Ohio. These were divided and the plants placed in thumb pots or in 3-inch pots December 21, 1935.

The bromegrass, Canada bluegrass, reed canary grass, and wirestem muhly were first potted in thumb pots December 21 and were kept in the cool greenhouse until January 30, 1936, when they were trans-

ferred to 3-inch pots. They were placed in the coldframe out of doors February 27, to remain until March 27, when they were transferred to 14-quart galvanized-iron buckets and to the tests beginning on that date.

The orchard grass and the three strains of bluegrass were first potted in 3-inch pots December 21, 1935, and remained in the cool greenhouse until March 16, 1936, when they were transferred to the galvanized-iron buckets and to the tests beginning March 27.

In one test *Poa bulbosa* was grown from bulbils formed normally in the inflorescence. These were sowed in flats in the cool greenhouse March 23, 1937, and germinated March 29; a clump of these plantlets was transplanted to buckets and placed in the tests April 23, when the plants were about 2 inches high.

In another test, clumps of overwintering *Poa bulbosa* were transplanted directly from the field to buckets March 25, 1937, and transferred to the tests March 29, when the shoots were only 1 inch high.

The Washington bent and the Metropolitan bent were transferred March 25, 1937, as small clumps, to buckets from the field at Arlington, Va., where they had passed the winter; the tests were begun on March 27. At that time the shoots were about 1 inch tall.

Material of *Muhlenbergia schreberi* was received from Ohio in February 1937 as large clumps, which were split up and potted to thumb pots February 23. They were later transferred to buckets and to the tests April 23, when the leafage was about 3 inches high.

Sorghastrum nutans and *Hystrix patula* were grown from seed obtained in the fall of 1937 from wild plants, the former from Little Cobbler Mountain and the latter from Bull Run Mountain, both in Fauquier County, Va. The seed was sown in flats in the coldframe December 16, 1937, which remained exposed to the normal cold of the wintertime until January 29, 1938, when the flats were brought into the cold greenhouse. Germination took place February 16 in the case of *Sorghastrum*, and February 4 in the case of *Hystrix*.

The *Sorghastrum* plants were placed singly in thumb pots March 23 and were transferred to buckets May 4, when 7 inches tall, three plants being grown in each bucket. The tests were begun on May 4.

The *Hystrix* plants were placed in thumb pots February 28 and transferred to pails May 3, when 4 inches tall, three plants being grown in each bucket. The tests were begun on May 3.

The material of the eastern gamagrass was obtained by dividing a large clump found near the Arlington Experiment Farm, Arlington, Va., and planting portions in buckets March 31, 1937. At this time shoots about 2 inches high were in evidence. The tests also were begun on that date.

It may be stated that with the exception of these grasses grown from seed, of which there were only two, *Hystrix* and *Sorghastrum*, all clumps of material represented clonal multiplication of selected strains. The plants were grown in 12- or 14-quart buckets with drainage holes in the bottom; one bucket was used for each test on a given treatment with the exception of the controls, represented by two buckets. The original clumps were taken from field material, and therefore experienced the normal outdoor conditions until divided and grown in the greenhouse in small pots, under temperatures of 50° to 55° F.

It is evident that all the tests within this range could not begin at the same time owing to seasonal differences in length of day; the 12-hour

day did not begin until near March 21, and days longer than that at later dates. For this reason the tests with the longer light intervals were begun when the natural length of day had reached the particular duration desired. Until that time the plants received the normally increasing daylight periods. The data in table 1, showing the constant daily light periods used and the date when the tests for each were begun, will make this clear.

TABLE 1.—*Length of the daily light periods used and dates when tests were begun*

Light period		Date tests were begun
Duration (hours)	Clock time	
10	6 a. m. to 4 p. m.	Mar. 27
12	6 a. m. to 6 p. m.	Do.
12.5	6 a. m. to 6:30 p. m.	Mar. 29
13	5 a. m. to 6 p. m.	Apr. 10
13.5	5 a. m. to 6:30 p. m.	Apr. 23
14	5 a. m. to 7 p. m.	May 6
14.5	5 a. m. to 9:30 p. m.	May 21

In all tests involving a range of 10 to 14.5 hours of light inclusive, natural daylight was used, darkness being secured by placing the plants in lightproof, ventilated houses each day. This was accomplished by keeping the buckets upon movable trucks mounted upon tracks. These trucks were moved in and out of the darkened houses, according to the schedules outlined above, to obtain each light period.

Since the constant light periods designated as 16 and 18 hours are in excess of the normal daylight periods of the Washington latitude, the use of supplemental electric light, from sunset on, was required to obtain these photoperiods. In these tests the added illumination was obtained from four 200-watt gas-filled tungsten lights with reflectors, mounted upon an iron frame that could be moved vertically to adjust the height of the lights, which were usually kept about 1 foot above the plants. These lights were arranged at the corners of a square the dimensions of which were 3 feet on a side from center to center of the lights. The intensity of the lights at this distance, as measured by a Weston illumination meter, model No. 1746, equipped with a Viscor filter to obtain as nearly as possible only visible radiation, amounted to about 300 to 400 footcandles.

Since the natural length of day is a variable, increasing to a maximum of about 14.9 hours at the summer solstice June 21, and decreasing at the same rate thereafter, constant light periods of 16 and 18 hours, respectively, required adjustments in the periods of artificial illumination after sunset to compensate for these seasonal increments and decrements of daylight throughout the growing season. This was done by the use of time switches, which were regulated at frequent intervals to maintain constant these periods of illumination.

One series of controls in buckets receiving the full seasonal length of day was maintained at the darkened house, while another similar series was maintained in the tests involving 16 and 18 hours of light, which were some distance away. The mean of these was taken as the representative figure in the tables. Plants of a number of the varieties that had overwintered in the field and also experienced the full length of day were also used for comparison.

The date of visible appearance of the first head from the boot, the date of pollination, the first noticeable evidence of yellowing or browning of the glumes or heads, and the date when about half the heads were straw-colored were noted for most of the varieties. The height of the tallest flowering stems was taken at the time pollen appeared. In some instances there was continued growth of the original flowering stem by branching, as in *Muhlenbergia mexicana* or *Bromus inermis*. In such cases later measurements sometimes showed greater lengths of stem under the column headed "Growth behavior" in the tables. Greater differences in this respect have often been shown where the natural day has been greatly extended by artificial light.

EXPERIMENTAL RESULTS AND DISCUSSION

CANADA BLUEGRASS

The behavior of Canada bluegrass (*Poa compressa*) (table 2) would indicate clearly that this species is a long-day type, since daylight periods of 10 hours to 13 hours inclusive were too short to allow flowering to take place. There was slight stem development, but no heads emerged in the tests of 12.5 and 13 hours. Although the plants receiving 13.5 hours of light flowered, there was some delay in the appearance of heads, showing that this length of day is near the upper limits of dominant vegetative expression. There was a particularly heavy growth of matted leafage where 10 and 12 hours of light were experienced. It is interesting to note that even where these plants experienced the full length of day at Washington, D. C., the bases of the stems were slightly decumbent, but with 16 and 18 hours of light, the stems were strictly erect. This is additional evidence that Canada bluegrass is an ecological form best adapted to very long days so far as flowering is concerned. As a matter of fact its range bears out this assumption, since it extends from Newfoundland and Alaska, southward to Georgia, Alabama, New Mexico, and California. At lower latitudes than this, this grass could be expected to reduce its flowering energies, or perhaps prove unable to flower, since lengths of day below 13.5 hours favor the dominance of vegetative expression. This grass appears to be well adapted to far northern regions (fig. 1).

TABLE 2.—*Responses of Canada bluegrass to different constant daily light periods*

Daily light period hours	Date when—				Tallest stem	Flower stems	Growth behavior
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored			
Hours					*Inches*	*Number*	
10	None	None	None	None	None	None	Heavy rosette growth; no stem elongation.
12	do	do	do	do	None	None	Do.
12.5	do	do	do	do	(1)	None	Slight stem elongation; very decumbent; no heads.
13	do	do	do	do	(1)	None	Do.
13.5	June 1	June 16	June 22	July 13	16	42	Somewhat decumbent at base.
14	May 25	June 11	June 19	do	15	81	Do.
14.5	May 20	June 8	do	July 6	17	147	Do.
16	May 12	June 1	June 11	June 18	24	138	Stems erect.
18	do	do	do	June 16	25	163	Do.
(2)	May 25	June 13	July 1	do	18	156	Stems somewhat decumbent at base.
(3)	May 5	June 12	June 30	July 17	14	64	Do.

[1] Slight. [2] Full length of day; plants in buckets. [3] Full length of day; plants in the field.

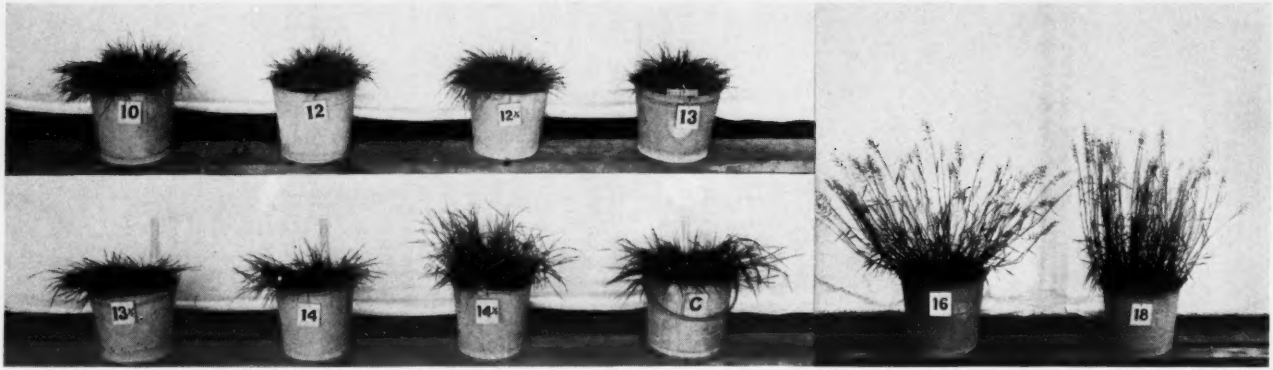

FIGURE 1.—Responses of Canada bluegrass (*Poa compressa*) to various constant daily photoperiods (hours) indicated. Control (C) received full length of day. There was no flowering until 13.5 hours of light each day were experienced, onlya few stems being produced on the 12- and 12.5-hour photoperiods. Dates of flowering with various photoperiods were as follows: With 13.5 hours, plants flowered June 16; 14 hours, June 11; 14.5 hours, June 8; full length of day (C), June 13; 16 hours, June 1; 18 hours, June 1. On the shorter lengths of day the stems were very decumbent, becoming strictly erect on the longer days. Canada bluegrass behaves as a typical long-day type of plant. Photographed May 29, 1936.

KENTUCKY BLUEGRASS

Three strains of Kentucky bluegrass (*Poa pratensis*) have been grown, one an early strain, one medium-late, and one a late strain (table 3).

From the dates of heading and the appearance of open florets liberating pollen, the early strain shows an advance of 3 to 4 weeks or more in heading over the medium-late and the late strains, the time of flowering also being earlier. The stems of the early strain were slightly decumbent in response to 10 hours of daylight, but were erect under all longer light periods (fig. 2).

TABLE 3.—*Responses of three strains of Kentucky bluegrass to different constant daily light periods*

EARLY STRAIN

Daily light period	Date when—				Tallest stem	Flower stems	Growth behavior
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored			
Hours					*Inches*	*Number*	
10	Mar. 2	Apr. 4	May 18	May 26	17	26	Stems slightly decumbent.
12	do	Apr. 14	May 20	May 20	15.5	22	Stems erect.
12.5	do	do	do	May 27	17	17	Do.
13	do	Apr. 29	do	May 29	20	16	Do.
13.5	do	Apr. 14	May 22	do	15.5	25	Do.
14	do	do	do	do	13.5	16	Do.
14.5	do	Apr. 27	May 19	June 5	16	13	Do.
16	do	Apr. 10	do	May 28	17	17	Do.
18	do	Apr. 16	May 18	May 27	15	17	Do.
(1)	do	do	do	do	17	18	Do.
(2)	do	Apr. 25	May 20	do	14.5	13	Do.

MEDIUM-LATE STRAIN

10	Mar. 25	Apr. 27	May 20	May 29	13	18	Stems very decumbent, lying almost flat upon the ground.
12	do	May 2	May 22	May 27	14	35	Do.
12.5	do	May 6	May 21	May 29	17	27	Stems less decumbent than in 10- and 12-hour tests.
13	do	Apr. 29	May 20	do	20	25	Stems more erect than for the 12.5-hour test.
13.5	do	May 4	do	May 28	20	20	Stems declined about as in the 12.5-hour test.
14	do	May 6	May 19	May 29	20	21	Stems more erect than for the 13.5-hour test.
14.5	do	May 7	May 21	June 4	13	22	Stems almost erect; only very slightly declined.
16	do	May 4	May 22	May 28	15	17	Stems strictly erect.
18	do	May 7	do	May 27	18.5	26	Do.
(1)	do	Apr. 28	May 18	May 28	15.5	23.5	Do.
(2)	do	May 4	May 25	May 29	17	13	Do.

LATE STRAIN

10	May 2	May 11	May 27	June 6	9	10	Stems somewhat declined.
12	Apr. 13	May 8	May 26	June 3	10	26	Stems nearly erect.
12.5	Apr. 15	May 4	May 21	June 4	12	19	Stems strictly erect.
13	do	May 8	May 23	June 3	11	30	Do.
13.5	Apr. 13	May 4	May 22	do	18	16	Do.
14	Apr. 15	May 7	May 23	do	15	22	Do.
14.5	Apr. 14	May 8	May 19	June 4	17	19	Do.
16	Apr. 20	May 11	May 25	June 11	18	15	Do.
18	Apr. 15	May 7	do	June 1	17	16	Do.
(1)	Apr. 13	May 5	May 19	June 3	16	23	Do.
(2)	Mar. 28	May 11	May 25	May 27	12	8	Do.

[1] Full length of day; plants in buckets.
[2] Full length of day; plants in the field.

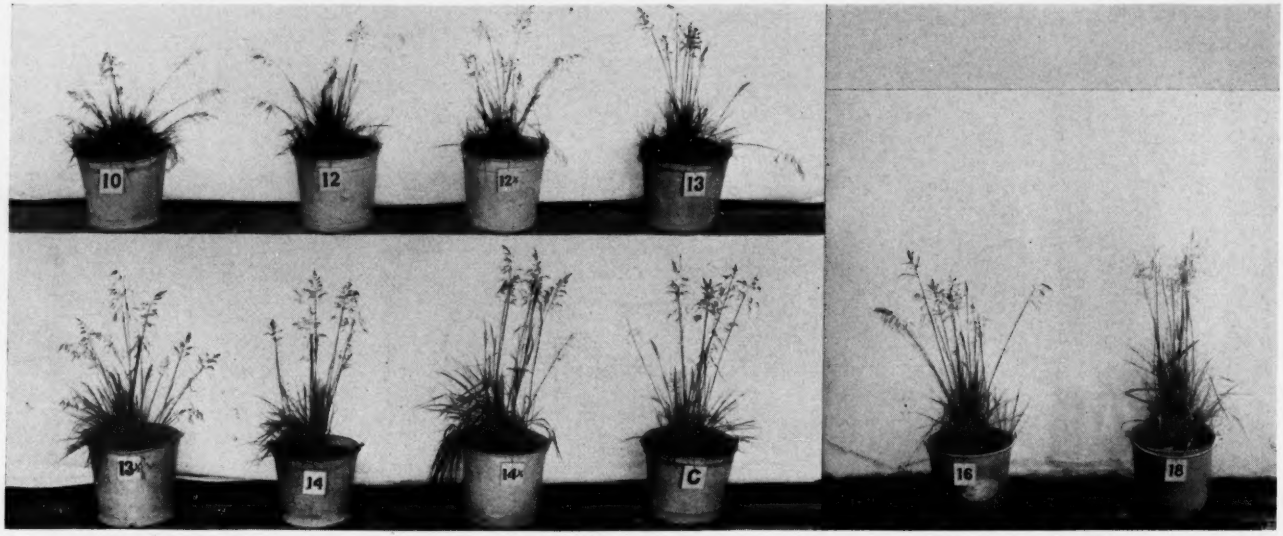

FIGURE 2.—Responses of early strain of Kentucky bluegrass (*Poa pratensis*) to various constant daily photoperiods (hours) indicated. Control (C) received full length of day. All the plants flowered readily. Dates of flowering with various photoperiods were as follows: With 10 hours, plants flowered April 4; 12 hours, April 14; 12.5 hours, April 14; 13 hours, April 29; 13.5 hours, April 14; 14 hours, April 14; 14.5 hours, April 27; full length of day (C) April 16; 16 hours, April 10; 18 hours, April 16. This early strain shows an indeterminate length-of-day behavior, the stems becoming more erect on the longer days. Photographed May 29, 1936.

The medium-late strain and the late strain of Kentucky bluegrass are less sharply defined in their responses than the early strain, yet in time of heading the medium-late strain is about 3 weeks earlier. This strain is rather distinctive, also, in that it shows very decumbent stems lying almost flat upon the ground when the daily light periods were maintained at 10 and 12 hours. The flowering stems of this strain did not become strictly erect until the full length of day and the 16- and 18-hour light periods were experienced.

The stems of the late strain of Kentucky bluegrass showed only a slightly declined growth in response to 10 hours of daylight, became nearly erect with 12 hours, and strictly erect with all longer light periods.

In the case of these three strains, the tendency toward the decumbent habit of growth does not appear to be associated entirely with length of day. The medium-late strain is perhaps inherently more of a prostrate type of grass than either the early or the late strain.

Kemp (*12*), of the Maryland Agricultural Experiment Station, has shown that the close grazing of pastures is an important biotic factor in the production of low and more or less prostrate types of grass and white clover. In such pastures he found the surviving types of bluegrass and orchard grass to be short and procumbent, as compared with the taller strains found in hayfields. In other words, a rigid natural selection has taken place, leaving only the smaller, more prostrate types to survive because they were better fitted to escape destructive close cropping.

While there may be a more or less inherent difference in the degree of expression of the prostrate habit of growth in the case of the three bluegrass strains here reported upon, the factor of length of day has been shown to modify greatly the expression of this growth form.

Kentucky bluegrass is of European origin, where the summer days are very long, and it is indicated that this grass is well adapted to the cooler northern regions, where long summer days prevail.

In the case of timothy strains, ranging by fairly uniform gradations from very early to very late in time of flowering, the stems become increasingly procumbent with increased lateness and required gradually longer daily light periods to attain erect growth.

Of the three strains of Kentucky bluegrass reported upon, the medium-late strain apparently has not followed this typical behavior of timothy, since it was more procumbent than the later strain. Further study of the various Kentucky bluegrass strains would be necessary to obtain a clear understanding of the differences of behavior between timothy and these bluegrasses.

ORCHARD GRASS

As the data indicate in table 4, orchard grass (*Dactylis glomerata*) is favored by the longer light periods and to that extent is a long-day type. Although weak flowering occurred in response to 12 hours of daylight, the stems were so declined as to lie practically flat upon the ground, a behavior quite exceptional as compared with the normal erect growth of this tussock-forming grass in the field.

This markedly declined habit of stem growth was also shown in response to the 12.5-, 13-, and 13.5-hour light periods, and the stems

did not become strictly erect until 14.5 hours of light had been experienced.

Ten hours of light gave rise to dense tussocks of leafage only.

It is interesting to note that the time of flowering and heading was but little affected through the series of constant daily light periods, thus indicating a rather abrupt change from the purely vegetative to the reproductive phase of growth occurring at some period between 10 and 12 hours of light.

The distribution of orchard grass extends into Newfoundland and Alaska on the north, ranging southward to near the southern limits of the United States. This range could be predicted upon the data presented in table 4 and figure 3.

TABLE 4.—*Responses of orchard grass to different constant daily light periods*

Daily light period	Date when—				Tallest stem	Flower stems	Growth behavior
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored			
Hours					*Inches*	*Number*	
10	None	None	None	None	None	None	Dense rosette of leaves; no stem elongation.
12	May 7	May 20	May 29	June 11	22	2	Two flowering stems only; so declined as to lie nearly flat upon the ground.
12.5	May 8	do	June 2	June 12	20	8	Stems so declined as to lie nearly flat upon the ground.
13	May 12	May 26	June 3	June 15	20	7	Do.
13.5	May 6	May 18	May 29	June 11	26	8	Stems somewhat more erect than at the 13-hour period yet strongly declined.
14	May 8	May 23	June 3	June 16	36	10	Stems almost erect.
14.5	May 6	May 21	June 4	do	35	12	Stems strictly erect.
16	do	May 15	May 28	June 3	38	34	Do.
18	May 9	May 25	do	do	28	22	Do.
(1)	May 8	May 21	June 1	June 15	35	16	Do.
(2)	May 7	May 23	June 2	June 18	31	16	Do.

[1] Full length of day; plants in buckets.
[2] Full length of day; plants in the field.

WIRESTEM MUHLY

The grass known as wirestem muhly (*Muhlenbergia mexicana*) shows nothing more distinctive in its day-length responses than that 10 and 12 hours of daylight each day cause very low growth, with the heads on the short stems appearing almost on the ground.

With increase of day length, the flowering stems become noticeably longer and strictly erect. By July 8 the longer photoperiods had produced stems 25 to 30 inches long, as compared with stems only 3.5 inches tall on the 10-hour day.

On the basis of its length-of-day responses and stem elongation, this grass is better adapted to the longer days than to extremely short days. However, there is probably an optimum temperature range to which the grass may respond, since in parts of the South it grows on the mountains rather than on the warmer lowlands (table 5).

FIGURE 3.—Responses of orchard grass (*Dactylis glomerata*) to various constant daily photoperiods (hours) indicated. Control (C) received full length of day. Plants receiving 10 hours of light were unable to flower. Dates of flowering with various photoperiods were as follows: With 12 hours, plants flowered May 20 (few heads); 12.5 hours, May 20; 13 hours, May 26; 13.5 hours, May 18; 14 hours, May 23; 14.5 hours, May 21; full length of day (C), May 23; 16 hours, May 15; 18 hours, May 25. Orchard grass behaves as a typical long-day plant, with a very low critical photoperiod inhibiting flowering. Photographed May 29, 1936.

TABLE 5.—*Responses of wirestem muhly to constant daily light periods*

Daily light period	Date when—				Growth behavior
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored	
Hours					
10	May 22	May 27	June 4	June 11	Growth very low; some heads appearing nearly on the ground, and could not be counted; tallest stem July 8 (3.5 in.) erect.
12	May 16	May 21	...do	...do	Do.
12.5	May 14	May 18	...do	...do	Same growth behavior as for 10-hour period; tallest stem July 8 (7 in.); erect.
13	...do	May 21	...do	...do	Same growth behavior as for 10-hour period; tallest stem July 8 (14 in.); erect.
13.5	May 13	May 18	...do	...do	Same growth behavior as for 10-hour period; tallest stem July 8 (17.5 in.); erect.
14	...do	May 19	...do	...do	Same growth behavior as for 10-hour period; tallest stem July 8 (18 in.); erect.
14.5	May 14	May 22			Same growth behavior as for 10-hour period; tallest stem July 8 (31.5 in.); erect.
16	May 9	May 21	June 11	June 18	Much new growth by nodal branching; no flowering from these; tallest stem July 8 (31 in.); strictly erect.
18	May 13	May 18	...do	June 13	Same growth behavior as for 16-hour period; tallest stem July 8 (27 in.).
(1)	May 16	May 20	June 8	June 8	Tallest stem July 8 (22 in.).
(2)	May 15	May 18	...do	...do	Tallest stem July 8 (5 in.)[3]

[1] Full length of day; plants in buckets.
[2] Full length of day; plants in the field.
[3] Dry conditions in the field may have operated to check growth in these plants.

NIMBLEWILL

The grass known as nimblewill (*Muhlenbergia schreberi*) is a native American species and, like *M. mexicana*, showed no very marked responses to the different photoperiods until artificial light was used to extend the length of day from sunset. With photoperiods of 16 or 18 hours, flowering was delayed a month or more as compared with those tests in which the natural day had been shortened by darkening. Only one or two heads appeared in these longer tests with artificial light, and a very characteristic prostrate growth of the stems was noticeable.

Muhlenbergia schreberi is a moisture-loving grass, and its range extends from the northern United States south to Florida, Texas, and eastern Mexico, and westward to Wisconsin and eastern Nebraska.

On the basis of its flowering responses under the various photoperiods presented in table 6, it is indicated that this grass is normally a native of the middle latitudes of the eastern half of the United States.

TABLE 6.—*Responses of nimblewill to different constant daily light periods*

Daily light period	Date when—				Tallest stem	Growth behavior
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored		
Hours					*Inches*	
10	May 21	June 2	June 25	July 12	14	Very fine, compact green growth.
12	May 25	...do	June 24	July 23	14	Do.
12.5	May 27	...do	...do	...do	20	Full flower, Aug. 11.
13	...do	June 21	...do	July 12	20	New growth and flowering, Aug. 11.
13.5	...do	June 2	June 30	July 15	21	Growing and flowering, Aug. 13.
14	May 26	June 9	July 3	...do	17	Heading continued, flowering, Aug. 13.
14.5	...do	...do	July 8	July 21	16	Green and full flower, Aug. 16.
16	Aug. 15	Aug. 19			25	Two heads only; growth green but flat on the ground.
18	July 15	July 18			30	One head only; stems prostrate.
(1)	June 7	June 10	June 21	Aug. 3	17	No new growth, Aug. 19; old growth browning.

[1] Full length of day; plants in buckets.

REED CANARY GRASS

Reed canary grass (*Phalaris arundinacea*) is quite plainly a long-day type of grass but is characterized by a rather low flowering limit as the days are decreased in length.

With 10 and 12 hours of light, the plants produced leafage only, and until 14 hours of light had been experienced few flowering stems arose and all were decumbent.

With 14.5 hours of light daily and with longer light periods, flower stems were produced more abundantly, these being erect or nearly so.

The time of flowering for the entire series of day lengths above 13 hours was very constant. The 12.5-hour test showed a delay of about 1 month, indicating that this was near the border line between the dominance of vegetative growth and the beginning of the flowering impulse. It is natural to believe that shorter light periods somewhere between the tests of 12.5 hours, where the plants flowered, though with delay, and 12 hours, where there were no indications of flowering, would have required still longer intervals to produce heads and flowering, until a point was reached where flowering ceased entirely.

The range of reed canary grass is mainly northern, extending to New Brunswick and Alaska. Southward its range appears to stop roughly on a line from North Carolina westward to California, reaching Oklahoma, New Mexico, and northern California.

This natural northern distribution is reflected in the response of the plants to the various increased daily light periods shown in the data of table 7 and in figure 4.

TABLE 7.—*Responses of reed canary grass to different constant daily light periods*

Daily light period	Date when—				Tallest stem	Flower stems	Growth behavior
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored			
Hours					*Inches*	*Number*	
10	None	None	None	None	None	None	Leafage only; no flower stems.
12	do	do	do	do	None	None	Do.
12.5	June 20	June 25	July 6	----------	28	1	Flowering stem somewhat decumbent.
13	May 23	June 1	----------	----------	27	1	Stem slightly decumbent.
13.5	May 25	May 28	June 29	July 6	21	(1)	Stem very decumbent, almost prostrate; one head May 25; shedding pollen June 16 at 20 in.
14	May 20	May 29	June 11	June 15	44	6	Stems very decumbent.
14.5	May 23	June 1	June 15	June 18	41	8	Stems mostly erect; only slightly declined.
16	May 19	May 26	May 28	June 11	46	8	Stems strictly erect; new stems forming July 8.
18	May 18	May 25	do	do	46	10	Do.
(2)	May 22	May 30	June 9	June 18	36	8	Stems mostly erect as in 14.5-hour day.
(3)	June 1	June 5	June 16	June 22	28	7	Stems rather noticeably declined.

[1] Several.
[2] Full length of day; plants in buckets.
[3] Full length of day; plants in the field.

SMOOTH BROMEGRASS

The data for smooth bromegrass (*Bromus inermis*) presented in table 10 indicate that we are dealing with a long-day type of plant.

In those tests involving the shorter light periods of 10, 12, 12.5, and 13 hours, dense leafage only was produced, or if stems formed they

FIGURE 4.—Responses of reed canary grass (*Phalaris arundinacea*) to various constant daily photoperiods (hours) indicated. Control (C) received full length of day. Plants receiving 10- and 12-hour photoperiods never developed stems. Dates of flowering with various photoperiods were as follows: With 12.5 hours, plants flowered June 25; 13 hours, June 1; 13.5 hours, May 28; 14 hours, May 29; 14.5 hours, June 1; full length of day (C), June 3; 16 hours, May 26; 18 hours, May 25. This species is a typical long-day plant in its responses, flowering and producing erect stems as the days are lengthened. Photographed May 29, 1936.

were few in number and so decumbent as to lie flat upon the ground.

With increase in the length of the daily periods of illumination, the number of flower stems increased, their length was augmented, and they became nearly or quite erect.

TABLE 8.—*Response of smooth bromegrass to different constant daily light periods*

Daily light period	Date when—				Tallest stem	Flower stems	Growth behavior [1]
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored			
Hours					*Inches*	*Number*	
10	None	None	None	None	None	None	Dense leafage only; no stems.
12	do	do	do	do	None	None	Do.
12.5	May 14	do	do	do	1	1	Mostly leafage; one stem, but head never emerged from sheath; stem very decumbent, flat upon ground.
13	May 6	May 23	do	June 22	9	1	Single flower stem flat upon ground; mostly leafage.
13.5	May 23	June 12	June 23	July 6	9	[2] 1 [3] 4	Stems very much declined.
14	do	June 6	do	July 1	15	14	Stems somewhat declined.
14.5	May 26	June 11	June 29	July 6	18	21	Stems more erect than at 14-hour period.
16	May 6	May 22	June 11	June 18	10	40	Stems erect; many new shoots evident June 2; 42 inches high.
18	do	May 23	do	June 16	18	40	Stems erect; many new shoots evident June 2; 33 inches high.
[4]	May 27	June 12	June 18	July 4	19	29	Stems somewhat decumbent at base.
[5]	May 9	June 1	July 18	July 10	10	40	Do.

[1] When these measurements do not agree with those shown in the preceding column, this is due to the fact that the former measurements were made at a later date, during which interval increase in height had taken place.
[2] Early.
[3] Late.
[4] Full length of day; plants in buckets.
[5] Full length of day; plants in the field.

This grass is cultivated successfully as a hay and pasture grass in the more northern States, from Michigan and Kansas to Washington and Oregon. It appears best adapted to the northern half of the United States, and is escaping rather freely in this area. Its length-of-day responses as revealed in the text appear to afford confirmatory evidence of this tendency toward a more northern distribution (fig. 5).

TIMOTHY

It would appear from the work of the British investigators Gregor (*10*) and Gregor and Sansome (*11*) and from that of Witte of Sweden (*14*), that the timothy populations of the cultivated species *Phleum pratense*, as commonly understood, include a number of strains or types differing in growth form and other characters.

In their analysis of these forms, Gregor and Sansome designated the "American" and European cultivated types as group 1. These workers recognized a second group, the "wild," which they called group 2. Both groups have more or less striking growth form and behavior characteristics in the field.

The basic (gametic) chromosome number in the genus *Phleum* appears to be 7. The members of group 1, as discussed by Gregor and Sansome, including the cultivated or hay grasses, are distinguished by having 42 somatic (6 *n*) chromosomes, while the forms of group 2 are diploid, having 14 somatic (2 *n*) chromosomes.

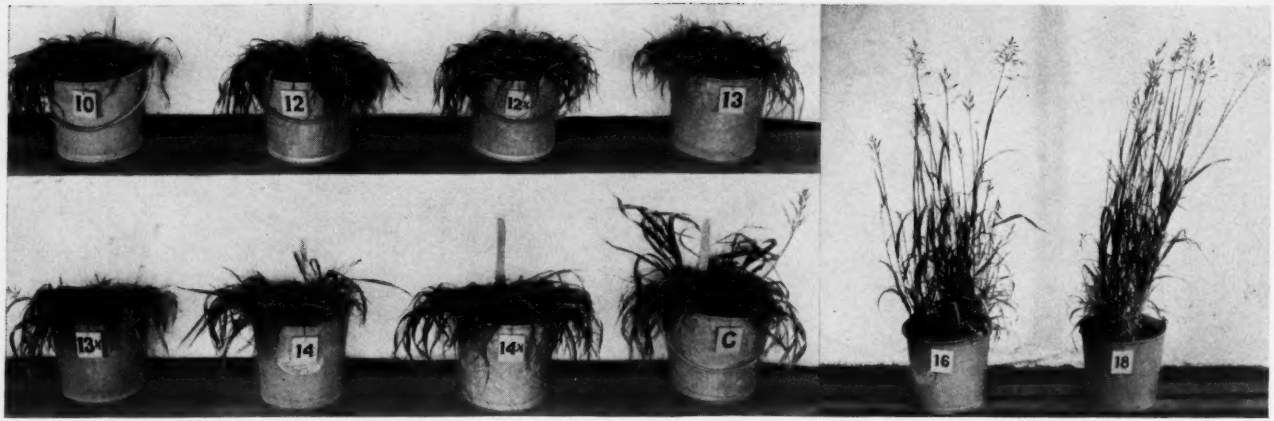

FIGURE 5.—Responses of bromegrass (*Bromus inermis*) to various constant daily photoperiods (hours) indicated. Control (C) received full length of day. Ten- and twelve-hour plants produced only leafage; 12.5-hour plants produced one decumbent head but did not flower. Dates of flowering with other photoperiods were as follows: With 13 hours, plants flowered May 23 (on one stem only); 13.5 hours, June 12; 14 hours, June 6; 14.5 hours, June 11; full length of day (C), June 8; 16 hours, May 22; 18 hours, May 23. This grass shows the behavior of a long-day plant. Photographed May 29, 1936.

It would appear that the earlier results presented by Evans and Allard (*7*) in 1934 were secured entirely with group 1, the hay varieties.

American Strains

The length-of-day responses of the two timothy strains F. C. 3937 (Huron) and F. C. 11901 (Marietta) are shown in table 9. Both

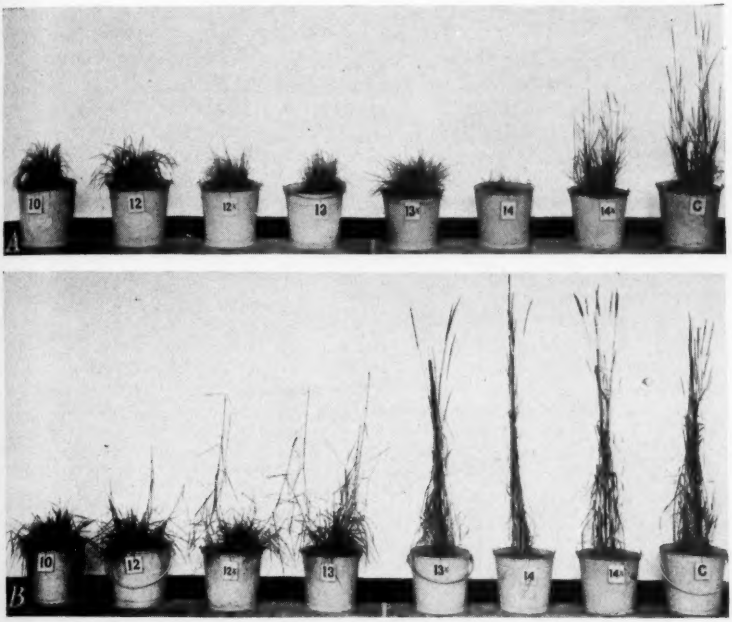

FIGURE 6.—Responses of two American strains of timothy to various constant daily photoperiods (hours) indicated, at Washington, D. C. Controls (C) received full length of day. *A*, Strain F. C. No. 3937 (Huron): Heading and flowering occurred only in response to lengths of day of 14.5 hours or longer; heads appeared in response to 14.5 hours of light June 28, and pollen appeared July 12. The full length of day (C) produced heads June 18 and pollen June 30. Photographed June 29, 1937. *B*, Strain F. C. No. 11901 (Marietta): There was weak decumbent stem growth even in response to 12 hours of light, flowering becoming normal with 13.5 hours of light and for all longer periods. Plants receiving 12 hours of light daily produced heads July 1; with 12.5 hours, heads appeared June 14 and pollen July 8; with 13 hours, heads appeared June 21 and pollen July 25; with 14 hours, heads appeared June 1 and pollen June 12; with 14.5 hours, heads appeared May 22 and pollen June 9. The full length of day (C) produced heads May 28 and pollen June 8. This strain has a much lower critical length of day for flowering than No. 3937, and for that reason is an earlier strain better adapted to more southerly sections of the timothy belt. Photographed June 28, 1937.

strains are of the early type. Of the two, 3937 (fig. 6, *A*) has a much higher critical length of day for flowering than 11901 (fig. 6, *B*), since the former was unable to flower in response to 13.5 hours of daylight each day, while the latter showed some indications of heading in response to daily light periods of 12 hours. However, the heads of F. C.

11901 showed excessive proliferation until daylight periods of 13.5 hours were experienced. The flower stems of both varieties became strictly erect in response to the full length of day of the Washington region, as well as the daily light periods of 16 and 18 hours that were obtained by the use of supplemental artificial light from sunset on.

TABLE 9.—*Responses of two American strains of timothy to different constant daily light periods*

Daily light period	Date when—				Tallest stem	Flower stems	Growth behavior
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored			
F. C. NO. 3937 (HURON)							
Hours					*Inches*	*Number*	
10	None	None	None	None	None	None	Heavy, dark-green leafage only.
12	do	do	do	do	None	None	Do.
12.5	do	do	do	do	None	None	Do.
13	do	do	do	do	None	None	Do.
13.5	do	do	do	do	None	None	1 stem; no heading Aug. 13.
14							Plant died July 24, at 7 inches.
14.5	June 28	July 12	Aug. 4	Aug. 16	38		
16	May 28	June 8	June 28	July 1	37		Stems erect.
18	May 23	do		July 10	38		Do.
(1)	June 18	June 30	Aug. 4	Aug. 13	45		Stems strictly erect.
F. C. NO. 11901 (MARIETTA)							
10	None	None	None	None		None	3 short stems, mostly leafage.
12	July 1	do			28	6	Inflorescence with excessive proliferation; no normal florets.
12.5	June 14	July 8	July 26		32		Inflorescence with much proliferation, remaining green Aug. 11.
13	June 21	None	July 25		36		Inflorescence with much proliferation.
13.5	June 5	June 16	July 19	July 30	50		No proliferation of inflorescence.
14	June 1	June 12	July 12	July 29	54	(2)	Do.
14.5	May 22	June 9	July 7	July 24	47	(2)	Do.
16	May 23	June 5	June 28	July 6	55	(2)	No proliferation of inflorescence; stems erect.
18	May 20	do	June 22	June 30	56	(2)	Do.
(1)	May 28	June 8	July 2	July 21	55	(2)	Do.

[1] Full length of day; plants in buckets.
[2] Many.

It is of interest to note that in response to the full length of day 3937 produced visible heads 21 days and pollen 22 days later than 11901. However, with increase of the daily light periods to 16 and 18 hours, there was no significant difference in the appearance of heads and pollen in the two strains.

While it is evident that both are well adapted to certain sections of the more northerly timothy-growing regions of the country, the day-length behavior as presented in table 9 would indicate that 11901 is an earlier variety, having a somewhat lower critical photoperiod for flowering, and for that reason would be better adapted as a hay grass to more southerly sections of the United States.

Evans has described both the Huron variety (*4*) and the Marietta variety (*5*).

EUROPEAN STRAINS

The day-length responses of the three European timothy strains discussed in the present paper—Harpenden (England), Russian

(Moscow), and Welsh strain S. 50—are of especial interest since, according to Prof. O. McConkey,[3] of Guelph, Canada, these are all diploid types.

These strains are rather distinctive in growth form, in that they are multitillering types, low-growing with decumbent stems, having the haplocorm (*3*) poorly developed or absent, and appear to be much better suited to severe grazing conditions, in latitudes where this type of timothy is well adapted, than are the upright-growing, less freely tillering hay grasses.

These three strains of timothy show very typically the normal responses of long-day plants, with a high critical photoperiod for the lower limits of flowering. As a matter of fact the Harpenden strain (table 10), originating in England, is distinctive in not being able to flower or in flowering with a sparse and abnormal growth in response to the longest days of the Washington, D. C., region, where the days are 14.9 hours long from sunrise to sunset at the time of the summer solstice.

Considering the Harpenden strain, it will be noted that the growth, even of leafage, was poor in response to 10 hours of daylight, there being no stem elongation. The growth of leafage improved as the daily light periods increased in length, but stems did not appear until the daily photoperiod had increased to 14.5 hours. Although there was a weak development of stems, these were so declined as to lie flat and stiffly upon the soil. This, however, is a characteristic behavior of these types, even in latitudes as far north as they can be grown.

TABLE 10.—*Responses of three European strains of timothy to different constant daily light periods*

HARPENDEN (ENGLAND) STRAIN

Daily light period	Date when—				Tallest stem	Flower stems	Growth behavior [1]
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored			
Hours					*Inches*	*Number*	
10	None	None	None	None	None	None	Growth poor; leafage only; no stems elongated.
12	do	do	do	do	None	None	Growth of leafage only; no stems elongated; better than at 10-hour period.
12.5	do	do	do	do	None	None	Growth of leafage only; better than at 12-hour period; no stem elongation.
13	do	do	do	do	None	None	Growth of leafage only; better than at 12.5-hour period; no stem elongation.
13.5	do	do	do	do	None	None	Good growth of leafage only; no stem elongation.
14	do	do	do	do	None	None	Do.
14.5	do	do	do	do	None	None	A few stems elongated, but so declined as to lie flat upon the soil.
16	May 26	June 11	June 25	.....	13	62	Poor stem growth, these very short, decumbent at base; heads small.
18	May 23	June 4	June 22	July 7	11	57	Better stem elongation, but decumbent at base.
(2)	July 11	July 20	Aug. 5	Aug. 16	10	27	1 or 2 stems only headed, but almost flat upon the ground.
(2)	None	None	None	None	None	None	Mostly leafage; 3 stems only, elongated and had not flowered July 15; stems very decumbent.

See footnotes at end of table.

[3] Oral statement.

TABLE 10.—*Responses of three European strains of timothy to different constant daily light periods*—Continued

RUSSIAN (MOSCOW) STRAIN

Daily light period	Date when—				Tallest stem	Flower stems	Growth behavior
	First heads appeared	Pollen appeared	First glumes were straw-colored	Half of heads were straw-colored			
Hours					*Inches*	*Number*	
10	None	None	None	None	None	None	Leafage only; no stem elongation.
12	do	do	do	do	None	None	Do.
12.5	do	do	do	do	None	None	Do.
13	do	do	do	do	None	None	Do.
13.5	do	do	do	do	None	None	Do.
14	do	do	do	do	None	None	Good growth leafage; no stem elongation; stems very decumbent.
14.5	June 18	June 29	Aug. 20		21	14	Very decumbent stems.
16	May 19	June 3	June 18	June 30	22.5	34	Good growth, but stems very decumbent.
18	May 16	June 1	do	do	27.5	35	Do.
(2)	June 18	June 29	July 24	July 3	22.5	53	Do.
(3)	do	do	July 20	Aug. 5	13	6	Stems decumbent at base.

WELSH STRAIN No. S. 50

10	None	None	None	None	None	None	Dense leafage only; no stem elongation.
12	do	do	do	do	None	None	Do.
12.5	do	do	do	do	None	None	Do.
13	do	do	do	do	None	None	Do.
13.5	do	do	do	do	None	None	Do.
14	do	do	do	do	None	None	Do.
14.5	July 6	July 30	July 30	Aug. 26 [4]	12	1	Stems (2) prostrate upon ground.
16	May 16	June 1	June 18		18	48	Flower stems very decumbent, prostrate.
18	do	May 28	do	June 30	19	59	Do.
(2)	June 18	June 29	July 30	Aug. 9	17.5	33.5	Flower stems more decumbent than for 16- and 18-hour day.
(3)	June 27	July 7	July 27	Aug. 12	16	17	Stems very decumbent at base for first 2 or 3 nodes.

[1] Where measurements, etc., do not agree with data of preceding columns, this is because these observations were made at later date, after changes had taken place.
[2] Full length of day; plants in buckets.
[3] Full length of day; plants in the field.
[4] Green.

Although flowering occurred in response to 16 and 18 hours of light, the stems were noticeably short, decumbent at base, and the heads small. However, the 18-hour light period stimulated a better and more normal growth of stems in length and erect habit than the 16-hour light period (fig. 7, *A*).

It is obvious that the Harpenden strain of timothy is adapted only to far northern latitudes of the United States and Canada, since in such latitudes the number and length of stems, and consequently hay production, would reach their maximum values.

Data in table 10 show that the Russian (Moscow) strain and the Welsh strain S. 50, from Canada, are very similar to the Harpenden strain in their length-of-day responses (fig. 7, *A*).

Both the Russian (Moscow) strain and the Welsh strain S.50 showed a somewhat better stem elongation than the Harpenden strain, but the stems of both for the most part were decumbent or prostrate although less so when the longer light periods of 16 and 18 hours were afforded the plants.

It is of interest to note that the longer light periods of 16 hours and of 18 hours have caused all three varieties to flower very noticeably

in advance of the controls experiencing the full length of day at Washington, D. C. This hastening of heading has amounted to as much as a month or more.

Since it would appear from the data presented in table 10 that the longest days of midsummer in the Washington region (lat. 39° N.) are

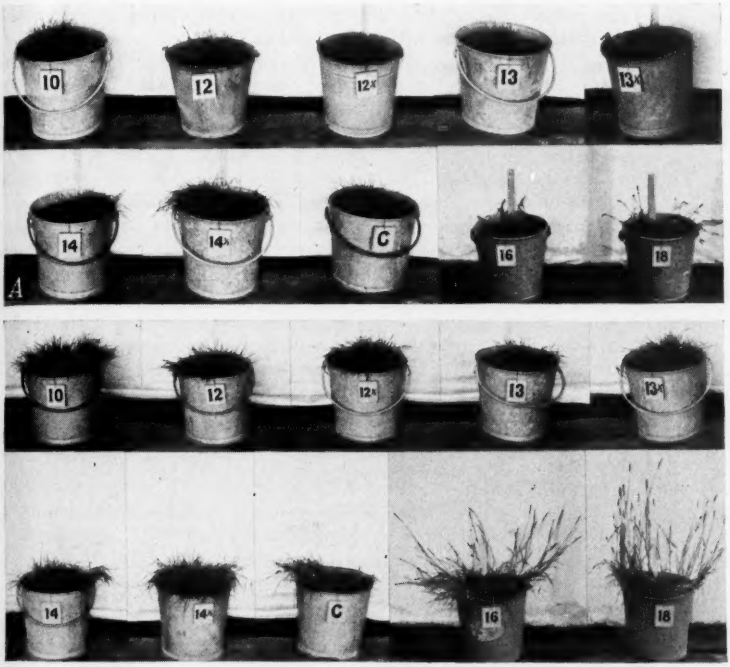

FIGURE 7.—Responses of two European timothy strains to various constant daily photoperiods (hours) indicated. Controls (C) received full length of day. *A*, Harpenden (England) strain. There was no stem growth or heading until the 14.5-hour photoperiod was experienced; then no heads were produced. Flowering took place as follows: With full length of day (C), plants flowered July 20 (very few heads); 16 hours, June 11; 18 hours, June 4. The stems of all were decumbent. This is a long-day timothy, requiring longer days than are experienced naturally in the Washington, D. C., region (lat. 39° N.). It is adapted to far northern latitudes. *B*, Welsh strain S.50, from Canada. There was no stem growth on the 10- to 14-hour photoperiods, inclusive. Nearly prostrate stems appeared on the 14.5-hour plants, but none headed. Dates of flowering with other photoperiods were as follows: With full length of day (C), plants flowered June 29; with 16 and 18 hours, May 16. The stems of all were very decumbent, those of the 18-hour plants being least so. This is a long-day strain, adapted only to the long days of high latitudes. Both photographed May 29, 1936.

scarcely above the critical lower photoperiod for flowering in these timothy strains, it is to be expected that flowering will be retarded as compared with that under greater lengths of day such as 16 hours or 18 hours. So long as the plants experience lengths of day below the critical length, purely vegetative expression must be dominant, and

sexual reproduction manifested by heading and flowering can only weakly assert itself or may remain entirely in abeyance.

These three timothy strains represent some of the latest yet tested in this country, and it is plain to see that this characteristic of lateness is dependent upon inherent behavior associated in part with length of day. With respect to stem elongation and flower formation, these strains must await the arrival of particular lengths of day before the reproductive mechanism is initiated or set in motion.

These late strains appear to have originated in England or on the continent of Europe and naturally have been selected to respond to lengths of day occurring in latitudes ranging from 50° to 60°, since southern England is just above the 50° parallel and Moscow, U. S. S. R., is just above the 55° parallel.

The northern boundary of the United States is wholly below latitude 50° N., and much of the best of the timothy-growing belt lies near 40° to 45°. On these parallels, the midsummer days from sunrise to sunset reach their maximum values of 15 hours and 15.3 hours respectively at the summer solstice, June 21. However, the plants experience these maximum lengths of day but briefly, owing to the ceaseless seasonal swing of the lengths of day between minimum values at the winter solstice and the maximum values of the summer solstice.

Data in table 11, showing the duration of the longest days at various latitudes, may be considered in connection with the behavior of the Harpenden strain of timothy, which in the Washington, D. C., region appears scarcely able to produce flowering stems or shows greatly delayed flowering.

TABLE 11.—*Hours from sunrise to sunset (intervals of 2 weeks) for latitudes* [1] *25° to 60°, during the longer days of the summertime*

[Summer solstice and maximum length of day, June 21]

Date	25°	30°	35°	39°	40°	45°	50°	55°	60°
April 21	12.53	13.04	13.17	13.28	13.31	13.48	14.09	14.34	15.08
May 1	13.07	13.21	13.37	13.52	13.55	14.17	14.42	15.15	16.00
May 11	13.18	13.35	13.54	14.11	14.16	14.41	15.14	15.55	16.50
May 21	13.28	13.47	14.09	14.29	14.35	15.04	15.41	16.29	17.83
June 1	13.36	13.58	14.22	14.44	14.50	15.24	16.04	16.58	18.20
June 11	13.40	14.02	14.30	14.52	14.58	15.33	16.19	17.18	18.44
June 21	13.42	14.04	14.32	14.56	15.02	15.38	16.24	17.24	18.54
July 1	13.40	14.02	14.30	14.53	14.58	15.34	16.18	17.18	18.36
July 11	13.36	13.58	14.22	14.45	14.50	15.24	16.04	16.58	18.19
July 21	13.29	13.48	14.12	14.31	14.36	15.06	15.44	16.33	17.41
Aug. 1	13.19	13.35	13.54	14.12	14.16	14.42	15.15	15.55	16.51
Aug. 11	13.07	13.20	13.38	13.51	13.55	14.19	14.43	15.18	16.01
Aug. 21	12.54	13.06	13.18	13.28	13.32	13.49	14.09	14.35	15.09

[1] Approximate geographical positions of various latitudes:
25° N.—Close to tip of southern Florida and central Mexico.
30° N.—Near the southern boundary of southern Louisiana and northern Florida.
35° N.—Near the southern boundary of North Carolina and Tennessee.
39° N.—Latitude of Washington, D. C.
40° N.—Near the southern boundary of Pennsylvania and central Ohio, Indiana, and Illinois.
45° N.—Near the center of Maine, northern Vermont, and Michigan, and southern Minnesota.
50° N.—Near northern France, southern England, central Quebec, and the northern boundary of the United States west of the Great Lakes.
55° N.—Near Moscow, U. S. S. R., southern Scotland and central Canada.
60° N.—Near Leningrad, U. S. S. R., southern Scandinavia, and southern Alaska.

It may, for example, be assumed that this strain is initiated into the flowering condition with a length of day near 15 hours. It is probable that a certain twilight intensity, at least in the evening, may also be operative, thus extending the effective length of day somewhat, for it is well established that quite low intensities when used to extend a

photoperiod of natural daylight beyond the critical length may be effective, as shown by Borthwick and Parker (*2*).

These authors have shown that, in the case of the Biloxi soybean, with a short day of 8 hours, which is below the critical photoperiod or the formation of flower primordia, an intensity of at least 100 footcandles of illumination must be supplied before flower primordia can arise. If, on the other hand, 8 hours of natural daylight are used and 8 additional hours of Mazda light are then added, making a total of 16 hours of continuous illumination, which is above the critical photoperiod for the initiation of flower primordia, the added light following the 8-hour period of natural light can be as weak as 0.6 footcandle, which appears to be near the lower limit of effective intensity under these conditions.

Kimball's (*13*) data would indicate that with practically cloudless skies the intensities of civil twilight,[4] beginning at dawn or ending in the evening when the true position of the sun's center is 6° below the horizon, range from about 0.4 footcandle to 33 footcandles. However, the case is somewhat different with the evening twilight, which at that time decreased from about 33 footcandles at sunset to 0.4 at the end of civil twilight. It is evident that practically all this period, amounting to about 35 to 36 minutes morning and evening through the summer season, is effective light in extending the length of day for the Biloxi soybeans.

With respect to the timothy strains under discussion, nothing is known of the actual lower limits of intensity required for flower-bud initiation. However, it may be assumed that both morning and evening twilight may be in part effective, and it is possible that the effective length of day from sunrise to sunset may be extended nearly an hour at Washington, D. C., lat. 39° N., by these two twilight periods.

For convenience 15 hours of illumination may be regarded as the lower limit for final flower development in the Harpenden strain, since heading and flowering were scarcely attained at Washington, D. C. Ignoring civil twilight, which is about 1 hour at Washington, and the effects of which cannot be evaluated for the long-day grasses at the present time, the plants do not receive 15 hours of light until about June 11; and even at the summer solstice the maximum period may not be much in excess of 15 hours of effective light. It will be noted from the data in table 13 that additional Mazda light after sunset, amounting to 200–300 footcandles and bringing the constant daily light periods to 16 and 18 hours, has caused heads to emerge May 26 and May 23, respectively, while the full length of day caused heads to appear July 11, about 7 weeks later. Since the tests began with these photoperiods April 21, about 1 month was required for visible heading, but actual initiation of the flower primordia must have taken place long before, perhaps within a few days from the beginning of the tests. If the rate of growth had been the same in response to the full length of day as in the 16-hour and 18-hour tests, the daily photoperiods of 15 hours, beginning about June 11, should have caused heading about July 11, as was observed.

Within the timothy belt more properly speaking, lying between

[4] The term "civil twilight" signifies that period after sunset and before sunrise during which there is enough light on clear days for ordinary occupations. Civil twilight ends at night and begins at dawn when the sun is about 6° below the horizon, and averages close to 35 to 36 minutes in length at Washington, D. C., for the year.

latitudes 45° and 50°, there is a noticeable increase in length of day, as shown in table 11. With respect to the original assumption of 15 hours as the shortest photoperiod capable of initiating sexual reproduction in the Harpenden strain, it may be noted that 15 hours of light (with twilight of about 1 hour or more additional) begins near May 11 for latitude 45° and that the daily light period remains above 15 hours until near August 1, i. e., it begins about 1 month earlier than for latitude 39° and persists about 1 month later. For latitude 50°, the comparable 15-hour photoperiod is advanced to near May 1; for latitude 55°, it is advanced to near April 21; and for 60°, nearer April 11, persisting until correspondingly later dates.

In a consideration of the ecological factor of length of day and its effects upon plant growth and reproduction in northern latitudes, it is important to consider these seasonal relationships brought about by changes from lower to higher latitudes. Not only may the critical length of day from sunrise to sunset be advanced to a much earlier date, thus giving a much longer period with lengths of day above the critical length before and after the summer solstice has arrived, but in very high latitudes the evening twilight lengthens appreciably, adding considerably to the effective length of day for some plants.

Thus it is plain why a timothy strain like Harpenden may become delayed in heading and flowering in the latitude of Washington, D. C. (lat. 39° N.), where the day length is perhaps unfavorable to the reproductive activities. At higher latitudes to which it has been adapted by selection and where lengths of day are perhaps much above the critical length for relatively long periods before the summer solstice, such a strain may become commercially valuable. Typical long-day plants, then, such as Harpenden, Russian (Moscow), and Welsh S. 50 strains of timothy, other conditions being favorable, are hastened rather than delayed in their flowering responses when carried to higher latitudes. This behavior is well shown when these strains have been subjected to constant daily light periods of 16 and 18 hours, affording photoperiods comparable to natural daylight durations experienced in latitudes 55° to 60°.

It is to be understood that the very decumbent growth form of these particular timothy strains is not related to a particular length of day, since this behavior is known to manifest itself in these forms as far north as they can be grown in Scotland and on the European mainland. While this expression appears to be a genetic character in these particular diploid strains, there is reason to believe that it is more or less modifiable, depending upon external environmental influences. There seems to be no logical reason why upright habit could not be developed by intelligent selection in some of these diploid strains.

It would appear that the biotic factor of intensive grazing has given these forms an advantage in pastures which they otherwise would not have, in competition with the more upright hay types.

BULBOUS BLUEGRASS

Bulbous bluegrass (*Poa bulbosa*) is unique in that some or all of the florets become converted into bulbils, a form of vegetative expression.

Tests to determine the response of *Poa bulbosa* to various lengths of day were made with small clumps from overwintering sod in the field and also with plantings of the small bulbils of the inflorescence.

The responses of the two groups were not the same. In the case of the overwintering sod material, there was no indication of flowering in any test and the plants appeared entirely brown as if mature and dead, from the shortest to the longest days, about the first of June.

The behavior of the young plants grown from the planted bulbils is shown in table 12. It will be seen from these data that stem and head formation, attended by the appearance of bulbils in the florets, did not occur until light periods of 13.5 hours were experienced. These bulbils continued to form for the most part in all photoperiods longer than 13 hours.

TABLE 12.—*Responses of bulbous bluegrass to different daily light periods*

Daily light period	Date when—		Tallest stem	Growth behavior
	First heads appeared	Pollen appeared		
Hours			*Inches*	
10	None	None	None	Leafage apparently dead, brown July 12.
12	do	do	None	Leafage apparently dead, brown July 29.
12.5	do	do	None	Leafage apparently dead, brown June 24.
13	do	do	None	Leafage apparently dead, brown July 3.
13.5	June 3		11	Small bulbils appeared in the heads and matured June 21. Plants apparently dead, brown, June 25.
14	None	None	7	Stems developed but no heads or bulbils; apparently dead, brown, June 25.
14.5	June 1		11	Small bulbils formed in the heads June 7, maturing June 30; apparently dead, brown July 9.
16	do		16	Small bulbils formed in the inflorescence; maturing June 15; plants apparently dead, brown Aug. 19.
18	May 28		16	Bulbils formed in inflorescence June 7; maturing June 21. Bulbils in this test much larger than in the test where 16 hours of light was used.
(1)	June 2		13	Small bulbils forming June 11; fully mature, brown June 21.

[1] Full length of day; plants in buckets.

The most striking difference observed in any test was associated with the light period of 18 hours, for the individual bulbils were strikingly large and even exceeded in size those formed with 16 hours of light. The tallest stems were formed in response to the 16-hour and the 18-hour photoperiods. By midsummer practically all the plants had become brown.

The behavior of the *Poa bulbosa* material was not as clearly expressed as has been the case with most grasses, and leaves much yet to be understood. Under the conditions of the tests reported there seemed to be no particular length of day when all the florets were sexually perfect, with functional stamens and pistils, since bulbils were always present.

Different strains of *Poa bulbosa* appear to behave differently with regard to the production of normal inflorescences, and these differences may have a genetic basis rather than representing simple response to some cultural condition.

CREEPING BENT

Two forms of a species of creeping bent (*Agrostis palustris*) have been studied, one known as Washington bent, the other as Metropolitan bent. These bents are used for lawns and putting greens. Both were studied from small clumps of overwintering field material.

The Metropolitan strain has shown a behavior rather different from that of the Washington strain. The former never produced heads in any test with photoperiods ranging from 10 hours to 18 hours; remaining green throughout the summer.

However, while there was no heading, growth was vigorous and stolons appeared in all the longer photoperiods, beginning with the 13-hour tests. It would appear that the longer light periods favor runner development, which is a prostrate type of vegetative stem, rather than the shorter photoperiods below 13 hours.

The behavior of the Washington bent (table 13) has been somewhat different, since this form was induced to flower on May 23 and 27, in response to 16 and 18 hours of light respectively. As a matter of fact, the plants flowered in no other test.

TABLE 13.—*Responses of Washington creeping bent to different constant daily light periods*

Daily light period	Date when—		Tallest stem	Growth behavior
	First heads appeared	Pollen appeared		
Hours			*Inches*	
10	None	None	None	A mat of short very compact green leafage only, Aug. 4.
12	do	do	None	A dark-green velvety growth of leafage only, Aug. 10.
12.5	do	do	None	A dark-green velvety growth of leafage only, Aug. 17.
13	do	do	None	A dark-green growth of leafage; short stolons appearing Aug. 11.
13.5	do	do	None	A dark-green growth of leafage; short stolons as in plants of 13-hour test Aug. 13.
14	do	do	None	Similar in growth to plants of 13.5-hour test.
14.5	do	do	None	Do.
16	May 23	June 9	16	Tips of heads browning July 15; half of heads brown July 22; plants green Aug. 19.
18	May 27	June 7	16	Tips of heads browning June 24; half of heads brown June 30; plants green Aug. 19.
(1)	None	None	None	Green leafage only, Aug. 18.

[1] Full length of day; plants in buckets.

The vegetative expression of the plants varied also, depending upon the photoperiod. While a dark-green velvety leafage was formed in response to all the tests where flowering did not occur, stolons became evident in response to 13 hours of light, as in the case of the Metropolitan strain, and were formed under all the longer photoperiods where a purely vegetative growth obtained.

INDIAN GRASS

One of the most interesting and beautiful grasses of the Washington region is the wild native Indian grass (*Sorghastrum nutans*). Aside from any consideration of usefulness in man's economies, this stately grass always commands attention in August, with its big, drooping, yellowish-brown, mobile panicles brightened with the rich yellow gold of the anthers.

Not only is it a conspicuous feature of our eastern wasteland successions, but it is a common constituent of the tall-grass prairies of the eastern Great Plains region (fig. 8).

In its length-of-day responses it has shown an almost classic behavior with the sharpness of its various responses to particular photoperiods.

Data in table 14 show that the plants remained strictly vegetative, with no stem development, until 13.5 hours of constant daily light had been experienced. On shorter photoperiods the buckets were filled with a dense leafage only, which, in the case of the 10-hour photoperiod, was very stiffly erect and had attained a height of only 7 inches in October as compared with 15 inches for the photoperiods of 12, 12.5, and 13 hours.

FIGURE 8.—Responses of Indian grass (*Sorghastrum nutans*) to various constant daily photoperiods (hours) indicated. Control (C) received full length of day. With 10- to 13-hour photoperiods plants did not flower but produced masses of stiff erect foliage. The critical photoperiod for flowering is near 13.5 hours; flowering occurred August 29. Plants receiving 14 and 14.5 hours and full length of day flowered August 20, August 29, and August 15, respectively. This grass appears to have a rather sharp lower critical photoperiod for flowering. Photographed August 31, 1938.

TABLE 14.—*Responses of Indian grass to different constant daily light periods*

Daily light period	Date when—		Tallest stem	Flower stems	Growth behavior
	First heads appeared	Pollen appeared			
Hours			*Inches*	*Number*	
10	None	None	None	None	Dense, stiffly erect leafage only, 7 inches high Oct. 2.
12	do	do	None	None	Dense leafage filling pail; 15 inches high Oct. 2.
12.5	do	do	None	None	Do.
13	do	do	None	None	Do.
13.5	Aug. 11	Aug. 29	46	10	Growth vigorous, normal.
14	do	Aug. 20	42	17	Do.
14.5	do	Aug. 29	64	21	Do.
16	Oct. 17	Oct. 20	56	9	Heads long remained in the boot, not heading till mid-October; of 13 stems only 9 produced heads.
18	Oct. 27	Oct. 31	50	6 (?)	Heads long remained in the boot, not heading till late October; of 22 stems only 6 produced heads.
(1)	Aug. 1	Aug. 15	56	19	Growth vigorous, normal.

[1] Full length of day; plants in buckets.

Heading and flowering occurred at approximately the same date in response to 13.5, 14, and 14.5 hours and to the full length of day.

In response to 16 and 18 hours of light, however, heading and flowering were delayed until late October, although the final heights

attained were about equal to those of the controls receiving the full length of day and to all other tests in which the plants flowered.

In the case of the 16-and 18-hour photoperiods, the heads long remained in the boot, indicating that these photoperiods and conditions were not entirely favorable to normal flowering. In the case of the 16-hour plants, a total of 13 stems developed, but only 9 headed and flowered; the 4 remaining did not appear to have heads in the boot. The plants experiencing the 18-hour photoperiod produced 16 sterile stems and only 6 flowering stems. It is obvious that these long photoperiods have not only greatly delayed anthesis, but have resulted in a large proportion of sterile stems or at least such as are incapable of maturing an inflorescence.

These plants were brought into the greenhouse October 12. At this season of the year the natural length of day is only 11.22 hours, and it is evident from the data of table 14 that this photoperiod is much too short for stem formation in this grass. Cessation of stem growth at this stage is probably incident to these unfavorable short photoperiods, since at this season the day length from sunrise to sunset is far below the critical photoperiod for stem formation and flowering, which is 13.5 hours in the various tests.

The natural range of this grass may now be considered in the light of the responses shown in the data of table 14.

The normal range of this species is from Maine, Quebec, and Manitoba, on the north, to Florida, Texas, Arizona, New Mexico, and Mexico, on the south. Roughly this grass finds its range near the northern and southern limits of the United States, embraced between latitudes 25° to 30° on the south and 45° to 50° on the north.

Since the tests have shown that a 13.5-hour photoperiod is favorable to flowering, it may be seen by reference to table 11 that 25° is very near the lower latitudinal limit of distribution of this grass, since the maximum length of the day is barely more than 13.4 hours.

Latitudes 45° and 50° have a mean day length considerably under 15.5 hours and above 16 hours, respectively, from May 21 to July 11. It is probable that latitude 50° is nearer the northern limit of distribution of *Sorghastrum nutans*, since the tests have shown that a photoperiod of 16 hours causes great delay in time of flowering.

These assumptions would only apply to the particular strain used in this test. It is always possible that the more northern material and the more southern material may include strains that are better adapted to the longer and shorter days, respectively, than is the particular type tested from middle latitudes.

In the more northern latitudes the factor of lower mean temperatures during the growing season will tend to work in the direction of furthering delay brought about by unfavorable lengths of day. In the case of such long-day plants as Indian grass, both factors combine to delay flowering and thus to define the northern limits of range. The southern limits will depend more purely upon the factor of the length of day alone, since the temperature means are increasing.

Since Indian grass is shown to be unable to flower or to flower with great delay when the days are either too short or too long, it may be considered to belong to that group of plants termed "intermediate" which the senior author has reported upon in a recent paper (*1*).

BOTTLEBRUSH

The genus *Hystrix*, to which bottlebrush belongs, is striking in having a very discontinuous distribution, one small group of two species being native to North America, another species native to Australia, and still another species native to Asia.

The eastern American species (*Hystrix patula*) is a woodland grass, characteristic of rocky slopes of the higher terrain. It flowers in June and contributes an element of beauty to its shaded woodland haunts with its bluish glaucous stems and long-awned, spreading florets loosely arranged in graceful spikes, but it has no known commercial value as a forage grass.

In its length-of-day response, it would appear to be a grass of the long-day class, since lengthened photoperiods favor its sexual reproduction, and the lower critical photoperiod for flowering is about 12 hours (table 15).

With only 10 hours of light daily, it produced mostly leafage and a single nearly stemless head among the leaves. With 12-hour daily photoperiods many stems were produced but these were short and some even failed to head.

TABLE 15.—*Responses of bottlebrush to different constant daily light periods*

Daily light period	Date when—		Tallest stem	Flower stems	Growth behavior
	First heads appeared	Pollen appeared			
Hours			*Inches*	*Number*	
10	June 20	None	None	None	One head practically stemless; mostly leafage.
12	June 16	June 30	15	(1)	Heads very low; many stems, some not heading.
12.5	June 7	June 21	17	(1)	All mature but dead Sept. 27.
13	June 15	July 1	25	(1)	Do.
13.5	June 13	June 30	30	(1)	Do.
14	June 21	July 1	30	(1)	Tops dead Sept. 27; small shoots below appear.
14.5	June 16	June 30	29	(1)	Green heads; small lateral green shoots appear.
16	June 13	June 25	26	(1)	Vigorous, normal growth.
18	June 9	...do.....	31	(1)	Do.
(2)	June 20	July 1	33	(1)	Do.

[1] Many.
[2] Full length of day; plants in buckets.

The length-of-day response of this grass, more especially its behavior in response to 16- and 18-hour photoperiods, would indicate a natural distribution well northward, and as a matter of fact its northern range extends at least to the northern boundary of the United States, near lat. 50° N. (fig. 9.)

EASTERN GAMAGRASS

Eastern gamagrass (*Tripsacum dactyloides*), whose length-of-day responses are shown in table 16, on the basis of its reactions to the various photoperiods, would appear to be indeterminate in its classification.

There has been no marked change in the time of flowering from the shortest photoperiods of 10 hours to the longest photoperiods of 18 hours. There appears to be some delay in heading and anthesis with the longer photoperiods, indicating that the shorter are a little more favorable to early sexual reproduction.

TABLE 16.—*Responses of eastern gamagrass to different constant daily light periods*

Daily light period	Date when—			Tallest stem	Growth behavior
	First heads appeared	Pollen appeared	Glumes browning		
Hours				*Inches*	
10	June 2	June 11	June 23	64	Both staminate and pistillate spikelets; fruitful.
12	June 4	June 14	June 21	56	Do.
12.5	June 7	...do...	...do...	45	Do.
13	...do...	...do...	June 30	67	Do.
13.5	...do...	June 15	June 23	55	Do.
14	June 20	June 28	July 10	56	Do.
14.5	June 21	...do...	July 7	60	Do.
16	July 13	July 18	July 24	63	Do.
18	June 23	June 30	July 6	54	Do.
(1)	June 21	June 28	July 3	65	Do.

[1] Full length of day; plants in buckets.

With respect to growth of stems in height and number and in the proportions of the unisexual staminate or pistillate florets, the responses of this species to the various photoperiods, whether long or short, were remarkably uniform.

On the basis of its readiness to grow and to flower normally with only 10 hours of light daily (fig. 10), it is indicated that this species is adapted to the length of day of tropical regions. As a matter of fact this grass in its southward range occurs in the West Indies, Mexico, and Brazil, the northern portion of which lies between lat. 5° N. and 0° (the Equator).

FIGURE 9.—Responses of bottlebrush (*Hystrix patula*) to various constant daily photoperiods (hours) indicated. Control (C) received full length of day. This grass shows little variation in response to different constant light periods, except at the lowermost limits of the shorter photoperiods. Plants receiving 10 hours of light daily finally produced a very weak panicle June 20; but florets produced no pollen. Dates of flowering with various photoperiods were as follows: With 12 hours, plants flowered June 30; 12.5 hours, June 21; 13 hours, June 15; 13.5 hours, June 13; 14 hours, June 21; 14.5 hours, June 16; full length of day (C), June 11. This grass, a native of the Washington, D. C., region, behaves somewhat as a long-day type with a very low critical photoperiod unfavorable to flowering. Photographed August 31, 1938.

The responses of this grass to the photoperiods of 16 and 18 hours would indicate that it should have a far northern distribution on the basis of the length-of-day factor alone, but in nature its northernmost limits appear to be Massachusetts, New York, Michigan, Illinois,

Iowa, and Nebraska. It is probable that other factors, such as low mean temperatures or great extremes of cold, may be a check to its northern distribution.

As the data stand, this species is indeterminate in its day-length responses, but with tendencies to delay flowering somewhat with very long photoperiods, indicating the more typical behavior of a short-day type of plant.

The genus *Tripsacum* is American and is represented by several species, most of which are limited to the extreme southern part of

FIGURE 10.—Responses of eastern gamagrass (*Tripsacum dactyloides*) to various constant daily photoperiods (hours) indicated. Control (C) received the full length of day at Washington, D. C. There was little difference in dates of flowering with various photoperiods: With 10 hours, plants flowered June 11; 12 hours, June 14; 12.5 hours, June 14; 13 hours, June 14; 13.5 hours, June 15; 14 hours, June 28; 14.5 hours, June 28; full length of day (C), June 28. At the time of flowering the heights of the plants were very constant, the mean being about 60 inches. The plants were photographed June 29, the earlier flowering plants of the shorter photoperiods elongating in the meanwhile, giving the difference in height shown in the picture. The tendency of this species to show delayed flowering on the longer photoperiods would indicate the more typical behavior of a short-day plant. Photographed June 29, 1937.

North America or to Central America, where some are cultivated for forage.

Eastern gamagrass is sporadic in its occurrence, preferring low, moist soils, and is not of great importance in American pastures.

SUMMARY

The length-of-day responses of *Poa compressa*, *P. pratensis* (3 strains), *P. bulbosa*, *Dactylis glomerata*, *Muhlenbergia mexicana*, *M. schreberi*, *Phalaris arundinacea*, *Bromus inermis*, *Phleum pratense* (5 strains), *Agrostis palustris* (2 strains), *Sorghastrum nutans*, *Hystrix patula*, and *Tripsacum dactyloides* were studied at the Arlington Experiment Farm at Arlington, Va.

The plants experienced the natural daylight for all photoperiods except those of 16 and 18 hours' duration, which required from sunset

the use of Mazda light from four 200-watt bulbs with reflectors, to supplement the normal length of day, giving an intensity at 1 foot, the distance at which the plants were maintained, of 300 to 400 foot-candles. Automatic time switches were used to turn the lights on and off at the proper time, these being adjusted from time to time to compensate for changes in the natural daylight period before and after the summer solstice.

For all constant photoperiods shorter than the full length of day, a portion of the daylight was excluded each day by keeping the plants in ventilated lightproof houses.

In the case of Canada bluegrass (*Poa compressa*), the critical photoperiod for heading and flowering is rather sharply delimited at 13.5 hours, while in the case of the very late timothy strain, Harpenden, flowering is attained with difficulty in response to the longest days (14.9 hours) of the Washington region.

The Kentucky bluegrass (*Poa pratensis*) strains, early, medium-late, and late, show more or less well-defined differences in growth behavior in their length-of-day responses, but under the conditions of the experiments flowering responses have not been so striking. The early strain, heading and flowering 3 to 4 weeks in advance of the two later strains, represents some inherent strain difference. Various degrees of the decumbent habit of stem growth were shown on the shorter day lengths by all the strains. It is evident that Kentucky bluegrass finds its best development, at least with respect to erect habit of growth, in the longer days and cooler temperatures of the more northern latitudes.

The time of flowering and heading of orchard grass (*Dactylis glomerata*) was but little affected by any of the photoperiods used, except that of 10 hours. This photoperiod was characterized by dense tussocks of leafage only.

Wirestem muhly (*Muhlenbergia mexicana*) showed nothing particularly distinctive in its responses to the various constant daily photoperiods with respect to time of heading and flowering. Ten hours of light daily caused very low growth, with flowering stems so short that they appeared almost on the ground. The longer photoperiods resulted mainly in greater elongation of the flower stems and a more erect habit of growth.

Nimblewill (*Muhlenbergia schreberi*) showed no very marked response to increased duration of the photoperiods until artificial light was used to extend the day to 16 hours and 18 hours, respectively. These photoperiods appeared to stimulate a very characteristic prostrate type of growth, and flowering was delayed a month or more.

Reed canary grass (*Phalaris arundinacea*) is a long-day grass, but its flowering extends down to include daily photoperiods only 12.5 hours in length. Flower stems were sparsely produced until 14.5 hours of light daily were experienced, and all were decumbent. With 10 and 12 hours of light daily the plants produced leafage only.

Smooth bromegrass (*Bromus inermis*) is likewise a long-day type of plant. There was no flowering in response to photoperiods of 10, 12, 12.5, and 13 hours, dense leafage only being produced. With increase of the daily photoperiod beyond 13 hours, flowering became more profuse and the stems longer and more erect in growth.

The various strains of timothy (*Phleum pratense*) have shown more or less marked differences in response to the different photoperiods. The American strains F. C. 3937 (Huron) and F. C. 11901 (Marietta)

are early flowering types. Of the two, 3937 has a much higher critical length of day for flowering than 11901, since the former was unable to flower in response to 13.5 hours, while the latter showed indications of heading at 12 hours. The flower stems of both became completely erect in response to the natural length of day at Washington, D. C., and to the longer photoperiods of 16 and 18 hours obtained by using artificial light. It is thus indicated that 11901 is an earlier variety, and for that reason is perhaps better adapted to more southern sections of the United States.

Three European strains of timothy were studied, i. e., Harpenden, Russian (Moscow), and Welsh strain S. 50. All show typically the behavior of long-day plants, with a high critical for the lower limits of flowering, and all are very similar in their length-of-day responses. These three timothy strains represent some of the latest yet tested in the United States, and are evidently best suited to high northern latitudes here, as well as in England or on the continent, where they originated.

Bulbous bluegrass (*Poa bulbosa*) was grown from bulbils and also from clumps taken from overwintering sod. The responses of these were somewhat different, since the sod material never flowered in any photoperiod. Young plants from bulbils did not produce flowers or bulbils until a light period of 13.5 hours was experienced, and these formed in all photoperiods longer than 13.5 hours.

An accentuation of stolon formation was found to be associated with longer days in the tests with the two creeping bents (*Agrostis palustris*), Metropolitan and Washington, and in the case of the Washington bent flowering was also associated with the longer days.

Indian grass (*Sorghastrum nutans*) is very sensitive to certain changes in length of day, the lower critical limit for flowering being about 13.5 hours. This species is greatly delayed in heading and flowering when the photoperiods have been increased to 16 or 18 hours. In its inability to flower in response to short days and in its greatly delayed or partially inhibited flowering with photoperiods of 16 hours or longer, this plant appears to be of the intermediate class in its flowering behavior, that is, there is complete or partial inhibition of flowering when the days are too short or too long.

Bottlebrush (*Hystrix patula*) is a woodland species, flowering most readily in response to long day and therefore finding its best development in middle and northern latitudes, as its natural range would indicate.

Gamagrass (*Tripsacum dactyloides*) is very unresponsive to changes in length of day, except when photoperiods are very long. This species grows particularly well in photoperiods as short as 10 hours, and this behavior is exemplified by its range southward into Mexico and into Brazil where truly equatorial lengths of day prevail.

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FURTHER STUDIES ON THE OAT SMUTS, WITH SPECIAL REFERENCE TO HYBRIDIZATION, CYTOLOGY, AND SEXUALITY [1]

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INTRODUCTION

Hybridization in the oat smut fungi has been demonstrated by a number of investigators (*2, 3, 7, 8, 10*)[2] and in some instances (*5, 7, 10*) pathogenically distinct races of *Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn. have been produced artificially by this process. In 1931, the writer (*3*) described a buff smut of oats which appeared in hybrid material but which later was found to have arisen through mutation in *U. levis* (*5*). It was further shown that the buff smut fungus crosses readily with *U. avenae* and *U. levis* and that the buff character is inherited as a recessive (*4, 6*). Consequently, in such hybrids a buff F_2 segregate is homozygous for this character. Therefore, by selecting a buff segregate on a variety immune from the buff parent and susceptible to the *U. avenae* or *U. levis* parent it is possible to obtain a race of the buff smut that possesses the pathogenicity of the other parent or of both parents. Such a hybrid race was reported by the writer (*5*) in 1936, and on this basis it appeared theoretically possible to produce, by hybridization, a race of the buff smut for every race of *U. avenae* and *U. levis* available. Accordingly, investigations were undertaken to determine the validity of this theory. Studies also have been made on nuclear behavior in the buff smut, on the inheritance of sorus type in two races of *U. avenae*, and on the process of sporidial fusion in all of the oat smuts. The results of these investigations are reported in this paper.

MATERIAL AND METHODS

The isolation of single sporidia and the determination of compatible combinations of monosporidial lines were accomplished by the methods previously described (*4*). Inoculations were made either by the method used formerly (*3*) or by the partial vacuum method described by Allison (*1*). Hybrid chlamydospores were obtained by inoculating Anthony (C. I.[3] 2143) oats with paired monosporidial lines of *Ustilago avenae* and the buff smut and *U. levis* and the buff smut. Because of the high degree of sterility in sporidia from hybrid chlamydospores (*3, 4*), the F_1 spores were used to inoculate differential varieties, and buff F_2 segregates were selected from varieties immune from the buff parent. Inoculum of succeeding generations of the F_2 buff selections was taken from the same varieties on which the F_2 segregate appeared.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 240.

[3] C. I. refers to accession number of Division of Cereal Crops and Diseases.

Two races of *Ustilago avenae* that produce distinctly different sorus types were used to study the heritability of this character. Hybrid spores were obtained in the manner described above, and since the sporidia from these F_1 spores will grow in culture, inoculations were made with combinations of monosporidial lines from the F_1 chlamydospores to obtain the F_2 population. Complete sets of four monosporidial lines were obtained from five F_1 chlamydospores; inoculations were made with the compatible combinations within each set, and one set was used in backcrosses to the parent lines.

The nuclei of the buff smut fungus in several stages of development were stained with Heidenhain's iron-alum heamatoxylin. The procedure described previously (*4*) was used, except that the material was destained with acid alcohol instead of iron-alum.

The process of sporidial fusion was studied by mating sporidia of opposite sex in pairs on plain water agar. The pairings were made with the aid of a Chambers' micromanipulator and the sporidia in each pair were distinguished from each other by difference in size, i. e., small sporidia were selected from one line and mated with relatively large sporidia selected from the other line.

RESULTS

PHYSIOLOGIC RACES OF BUFF SMUT

Seven races of the buff smut have been identified on the basis of the differential reaction of certain oat varieties. One of these races arose through mutation, one represents a field collection, and five were produced by hybridization. The results of pathogenicity tests on which the separation of the races was based are presented in table 1. The origin and distinguishing characteristics of each race are as follows:

Race 1.—Mutant from *Ustilago levis* collected in McLeod County, Minn., in 1930. As shown in table 1, Canadian is highly susceptible to this race and 17 percent smut was produced on Richland.

Race 2.—An F_2 segregate on Gothland from a hybrid between buff race 1 and a Gothland race of *Ustilago avenae*. Differs from race 1 primarily by the susceptible reaction of Gothland to race 2.

Race 3.—Collected by Harland Stevens from a row of oats in a seed stock nursery grown for T. R. Stanton at Aberdeen, Idaho, in 1935, the seed of which was obtained from a Chicago, Ill., grain elevator. This race differs from race 1 by the susceptible reaction of Gothland and from race 2 by the susceptibility of Richland.

Race 4.—An F_2 segregate on Black Mesdag from a hybrid between buff race 1 and a Black Mesdag-Monarch race of *Ustilago levis*. This race is characterized by its capacity to infect Black Mesdag.

Race 5.—An F_2 segregate on Monarch from a hybrid between buff race 2 and a Black Mesdag-Monarch race of *Ustilago levis*. Its virulence on Gothland, Monarch, and Black Mesdag distinguishes this race from the others. It is notable that Monarch is more susceptible and Black Mesdag is less susceptible to this race than to the *U. levis* parent and that Gothland is less susceptible than it is to the buff parent (table 1).

Race 6.—An F_2 segregate on Trojan from a hybrid between buff race 1 and a Monarch-Richland race of *Ustilago levis*. This race is outstanding for its capacity to infect Alabama Red Rustproof, which is resistant to both parent races. It is definitely less virulent on Monarch than the *U. levis* parent and about equal in virulence to both parent races on Richland.

Race 7.—An F_2 segregate on Fulghum from a hybrid between buff race 1 and a Monarch-Fulghum race of *Ustilago levis*. This race is characterized by its virulence on Fulghum and Monarch and, to a slight degree, on Black Mesdag.

TABLE 1.—*Differential host reaction to parent and hybrid races of buff smut of oats*

Parent and hybrid races of oat smut	Race No.	Percentage of smut in host testers—						
		Canadian (C. I. 1625)	Gothland (C. I. 1898)	Monarch (C. I. 1876)	Black Mesdag (C. I. 1877)	Richland (C. I. 787)	Fulghum (C. I. 708)	Alabama selection Red Rustproof (C.I.1355)
Ustilago levis parent		100	0		0			7
Buff mutant race	1	84	0	0	0	17	0	0
U. avenae parent		98	77	6	0	0	0	0
Buff mutant parent		84	0	0	0	17	0	0
Buff hybrid race	2	85	58	0	0	3	0	0
Buff field race	3	87	66	1	0	29	3	0
U. levis parent		100	0	24	83	0	0	0
Buff mutant parent		84	0	0	0	17	0	0
Buff hybrid race	4	82	0	2	75	0	0	0
U. levis parent		100	0	24	83	0	0	0
Buff hybrid parent		85	58	0	0	3	0	0
Buff hybrid race	5	92	39	91	25	0	1	0
U. levis parent		100	0	67	0	34	0	0
Buff mutant parent		84	0	0	0	17	0	0
Buff hybrid race	6	80	0	21	0	20	0	55
U. levis parent		87	0	72	33	0	79	0
Buff mutant parent		84	0	0	0	17	0	0
Buff hybrid race	7	83	0	94	13	0	54	0

It is apparent from the foregoing results that new races of the buff smut may be produced at will by crossing any race of this fungus with races of *Ustilago avenae* and *U. levis*. A new race of the buff smut was obtained from every cross made between races of the buff smut and different races of these two species. In some cases the new races are similar in pathogenicity to the *U. avenae* or *U. levis* parent, while in others the virulence of the hybrid race is greater than that of either parent. For example, race 2 and its *U. avenae* parent both infect Gothland while all other varieties are resistant, and race 7 and its *U. levis* parent both infect Monarch, Black Mesdag, and Fulghum. Race 4, however, infects Black Mesdag only, whereas the *U. levis* parent infects Monarch in addition to Black Mesdag. On the other hand, race 5 infects all of the varieties (Gothland, Monarch, Black Mesdag) that the two parent races infect; and race 6, like its *U. levis* parent, infects Monarch and Richland but, unlike either parent, can infect Alabama Red Rustproof. Just how the infective capacity of race 6 for Alabama Red Rustproof arose cannot be explained by the data at hand.

Race 1 infects Canadian and, to some extent, Richland, and apparently it has the same pathogenicity as the *Ustilago levis* race from which it mutated. It is notable, however, that in earlier tests Monarch also was infected by this race (*5*). It would appear, therefore, that buff race 1 originally was heterozygous for pathogenicity and those biotypes capable of infecting Monarch were lost by selective elimination on another variety. Similar results have been obtained with race 2. Gothland and Monarch were infected by this race in the F_3 (*5*), whereas only Gothland was infected in the F_8 (table 1). Sampson and Western (*9*) have shown that there is a definite selective influence of the host on the relative stability of physiologic races of the oat smut fungi. They pointed out, however, that it would be theoretically possible for a heterozygous condition to persist through several chlamydospore generations, which would limit the efficiency of screening as a means of obtaining races pure for patho-

genicity. Possibly this was the case with race 2, and the repeated selection of inoculum from Gothland was necessary to gradually eliminate the capacity for infecting Monarch. Little is known, however, regarding the stability of such hybrid races of the oat smuts. The majority of the races listed in table 1 (races 4, 5, 6, and 7) are in the F_4 generation, and if inoculum of future generations is taken from the same variety then the pathogenicity for certain other varieties may become lost. This would be highly probable in the case of a hybrid race that carries the pathogenic properties of both parents, such as race 5 (table 1). Race 5 was obtained by selecting a buff F_2 segregate on Monarch, and inoculum has been taken from the same variety in succeeding generations. Repeated selection of inoculum of this race from Monarch, however, might finally eliminate the Gothland and Black Mesdag pathogenicity, or the selection of inoculum from Gothland might result in the loss of the Monarch and Black Mesdag virulence. On the other hand, if there were linkage of factors for virulence on these varieties, or if this virulence were controlled by a single factor, both of which considerations seem improbable in this case, then the reaction would remain constant in future generations regardless of the variety from which inoculum might be obtained, unless the linkage were to become broken or mutation were to occur.

Similar results might be expected with races 6 and 7, while race 4 probably will remain constant in virulence if the inoculum is always taken from Black Mesdag. Therefore, in the light of these considerations, it is possible that the host reaction of the buff smut races shown in table 1 will change to some extent by continuous propagation on specific varieties. Considering the pedigree of these races, however, it seems improbable that such changes as may occur will materially affect their differentiation.

It will be noted in table 1 that comparative tests with the parent or parents of race 3 were not made, the reason being that its pedigree is not known. This race was discovered in 1936 among a large number of collections of *Ustilago avenae* and *U. levis* obtained from the 1935 seed stock nursery at Aberdeen, Idaho. The row from which this buff smut specimen was obtained had been sown to seed collected in a grain elevator in Chicago, Ill. Eleven smutted panicles were in the collection, of which four were *U. avenae*, four *U. levis*, and three buff smut. No other specimens of buff smut were found in 1,877 smutted panicles examined, all of which came from the seed stock nursery mentioned above. This is the only record of the occurrence of the buff smut under natural conditions. Furthermore, the record would seem to preclude any possibility that this instance of natural occurrence of the buff smut was due to an escape from the experimental material, especially in view of the fact that the panicles smutted with race 3 are characteristically different from those of race 1, the original buff smut, or any of the hybrid races. Race 1 produces sori entirely covered by the outer glumes, while race 3 completely destroys the outer glumes, leaving the sori exposed. The other races, for the most part, partially destroy the outer glumes, thus producing a type of smutted panicle intermediate between the two extremes of races 1 and 3. The spores of the buff smut, like

those of *U. levis*, remain intact in the sorus and are not readily disseminated as in *U. avenae*. Since the original buff smut race arose by mutation (*5*) it seems probable that race 3 is the result of a recurring mutation in *U. levis* that took place under natural conditions. Consequently, the buff smut fungus might justifiably be recognized as a variety of *U. levis* or possibly even as a distinct species.

CYTOLOGY

The results of studies on nuclear behavior in the buff smut fungus indicate that this phenomenon is fundamentally the same as in *Ustilago avenae* and *U. levis*. The nuclear condition in the various stages of development that were observed is shown in figure 1. The mature chlamydospore contains a single diploid nucleus (fig. 1, *A*). Meiotic division of this nucleus accompanies spore germination and one nucleus becomes located in each cell of the promycelium (*B*). The promycelium occasionally has four cells but usually three, the spore functioning as the fourth cell. Mitotic division of the nuclei of the promycelium accompanies the budding of a sporidium from each cell and one nucleus passes into each sporidium, the other remaining in the promycelial cell from which other sporidia may bud (*C*). Each sporidium contains a single haploid nucleus, which divides when the sporidium buds and one nucleus passes into the daughter sporidium. Binucleate sporidia frequently are observed, in which case it is presumed that nuclear division preceded sporidial budding (*D*). When sporidia of opposite sex fuse, the nucleus from one passes through the copulation tube into the other, thus initiating the dikaryophase (*E*). Each pair of fused sporidia produces an infection hypha into which the paired nuclei pass (*F*). Presumably the binucleate condition persists throughout the parasitic stage, nuclear fusion occurring when the spores become mature. If this presumption is correct, then nuclear behavior in the buff smut is identical with that of *U. avenae* and *U. levis*.

INHERITANCE OF SORUS TYPE

Two types of smutted panicles produced by two races of *Ustilago avenae* were observed in one row of Victory (C. I. 560) oats in the physiologic race nursery at Pullman, Wash., in 1937. Most of the smutted panicles in this row had the usual brown powdery type of sorus. A few panicles, however, had a distinctly black indurate type of sorus, and the spores of this type were slightly darker in color and less prominently echinulate than those of the powdery type. In both types the glumes had been completely destroyed, as shown in figure 2. The results of inoculations with spores from the two types of sorus indicated that these characters were genetically distinct. Studies were undertaken, therefore, to determine the nature of their inheritance. Combinations of monosporidial lines were used for inoculum, and seed of Anthony (C. I. 2143) oats was inoculated and grown to maturity in the greenhouse for the F_1 and in the field for the F_2. The results of these studies are summarized in tables 2, 3, and 4.

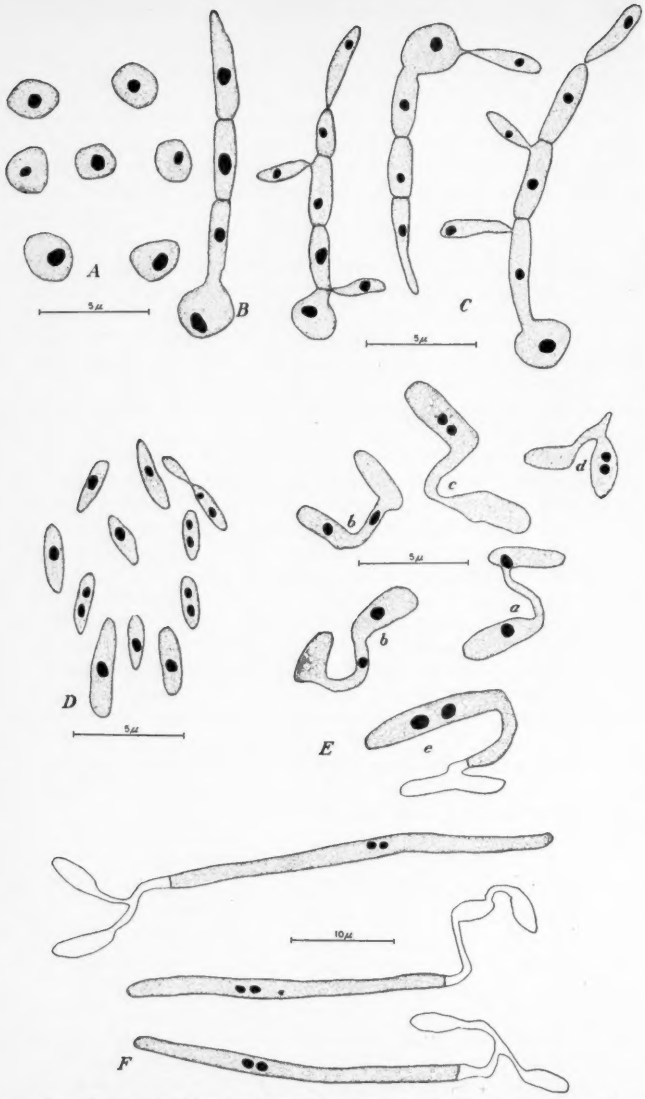

FIGURE 1.—Camera-lucida drawings showing the nuclear condition in various stages of development of the buff smut fungus. *A*, Mature chlamydospores. *B*, Germinated chlamydospore prior to sporidial formation. *C*, Germinated chlamydospores with sporidia budding from the promycelial cells. *D*, Haploid uninucleate and binucleate sporidia. *E*, Pairs of conjugated sporidia showing nuclei in various stages of migration: *a*, Nucleus about to enter the fusion tube; *b*, nucleus passing through the tube; *c*, binucleate stage or dikaryophase resulting from the migration of the nucleus from one sporidium to the other; *d*, beginning the production of the infection hypha following initiation of the dikaryophase; *e*, binucleate stage in which nuclear migration was accompanied or followed by migration of the cytoplasm into the sporidium which contains the nuclei. *F*, Infection hyphae.

FIGURE 2.—Smutted panicles of Victory oats showing two types of sorus produced by two physiologic races of *Ustilago avenae*. *A*, Powdery type, which sheds spores readily. (Note spore masses that were shed when the panicles were tapped against the background.) *B*, Indurate type, which does not shed spores.

TABLE 2.—*Type of sorus produced on Anthony oats by two races of Ustilago avenae and hybrids between them*

Parent sorus type	Chlamydospore No.	Number of sporidial combinations	Type of sorus produced by—	
			F_1	F_2
Powdery	56	4	Powdery	Powdery.
	57	4		
	58	4		
Indurate	54	4	Indurate	Indurate.
	55	4		
	59	2		
	60	2		
Powdery and indurate	56×54	8	Powdery	Powdery and indurate.
	57×55	8		
	58×59	6		
	58×60	6		

TABLE 3.—*Segregation of factors* [1] *for type of sorus in crosses between two physiologic races of Ustilago avenae*

F_1				F_2		
Chlamydospore No. and genotype	Sporidium No.	Sex [2]	Gametes	Genotype	Ratio	Type of sorus
93 (*Pp*)	1	+	*p*	*PP*	1:2:1	Powdery.
	2	+	*P*	*Pp*		Do.
	3	−	*P*	*Pp*		Do.
	4	−	*p*	*pp*		Indurate.
94 (*Pp*)	1	+	*P*	*Pp*	4:0	Powdery.
	2	−	*p*	*Pp*		Do.
	3	+	*P*	*Pp*		Do.
	4	−	*p*	*Pp*		Do.
95 (*Pp*)	1	+	*p*	*PP*	1:2:1	Powdery.
	2	−	*P*	*Pp*		Do.
	3	+	*P*	*Pp*		Do.
	4	−	*p*	*pp*		Indurate.
96 (*Pp*)	1	+	*P*	*PP*	1:2:1	Powdery.
	2	+	*p*	*Pp*		Do.
	3	−	*P*	*Pp*		Do.
	4	−	*p*	*pp*		Indurate.
97 (*Pp*)	1	+	*P*	*Pp*	4:0	Powdery.
	2	+	*P*	*Pp*		Do.
	3	−	*p*	*Pp*		Do.
	4	−	*p*	*Pp*		Do.

[1] *P*=powdery; *p*=indurate.
[2] Plus (+) and minus (−) signs indicate sporidia of opposite sex.

TABLE 4.—*Results obtained by backcrossing sporidia from a hybrid chlamydospore with sporidia from the parent chlamydospores to determine the heritability of sorus type*

USTILAGO AVENAE 94 (Pp) × U. AVENAE 56 (PP)

U. avenae 94 (*Pp*) hybrid			*U. avenae* 56 (*PP*) parent			Backcross	
Sporidium	Sex	Gamete	Sporidium	Sex	Gamete	Genotype	Type of sorus
1	+	*P*	2	−	*P*	*PP*	Powdery.
1	+	*P*	3	−	*P*	*PP*	Do.
2	−	*p*	1	+	*P*	*Pp*	Do.
2	−	*p*	4	+	*P*	*Pp*	Do.
3	+	*P*	2	−	*P*	*PP*	Do.
3	+	*P*	3	−	*P*	*PP*	Do.
4	−	*p*	1	+	*P*	*Pp*	Do.
4	−	*p*	4	+	*P*	*Pp*	Do.

USTILAGO AVENAE 94 (Pp) × U. AVENAE 54 (Pp)

U. avenae 94 (*Pp*) hybrid			*U. avenae* 54 (*pp*) parent			Backcross	
Sporidium	Sex	Gamete	Sporidium	Sex	Gamete	Genotype	Type of sorus
1	+	*P*	2	−	*p*	*Pp*	Powdery.
1	+	*P*	3	−	*p*	*Pp*	Do.
2	−	*p*	1	+	*p*	*pp*	No infection.
2	−	*p*	4	+	*p*	*pp*	Indurate.
3	+	*P*	2	−	*p*	*Pp*	Powdery.
3	+	*P*	3	−	*p*	*Pp*	Do.
4	−	*p*	1	+	*p*	*pp*	No infection.
4	−	*p*	4	+	*p*	*pp*	Indurate.

As shown in table 2, the 12 monosporidial combinations representing three chlamydospores from the powdery type of sorus produced that type of sorus in the F_1 and F_2, and the 12 monosporidial combi-

nations representing four chlamydospores from the indurate type produced that type of sorus. Apparently, therefore, the chlamydospores from which the monosporidial lines were obtained were homozygous for sorus type. The 28 combinations between monosporidial lines from the two types of sorus produced powdery sori in the F_1, indicating dominance of the factor for this type of sorus. In the F_2, there was segregation into powdery and indurate sorus types. The manner in which segregation of factors for the two types of sorus occurs is shown by the results from inoculations with crosses between monosporidial lines obtained from five F_1 chlamydospores (table 3), and by backcrossing the monosporidial lines from one F_1 spore with four monosporidial lines from one chlamydospore of each parent (table 4). As shown in table 3, there was independent segregation of factors for sex and sorus type in three of the chlamydospores (93, 95, 96), and the sorus type appeared in a ratio of 3 powdery to 1 indurate. The genotype ratio was found to be 1 : 2 : 1, as indicated. In chlamydospores 94 and 97, segregation of factors for sex and sorus type occurred in the same nuclear division and, therefore, all of the sori were powdery, the ratio being 4 : 0, as shown. Theoretically, these F_2 chlamydospores are heterozygous and should produce both types of sorus in a 3 : 1 ratio in the F_3, if segregation of factors for sex and sorus type occurs independently. In the backcrosses with the powdery parent (*Ustilago avenae* 56, table 4) all of the combinations produced powdery sori, as expected, since this character is dominant. The spores of half of these backcrosses were determined to be homozygous for the powdery sorus and the other half heterozygous. In the backcrosses with the indurate parent, four of the crosses produced powdery sori the spores of which were heterozygous, two crosses produced indurate sori the spores of which were homozygous, and two crosses failed to infect. Presumably the two crosses that failed to infect would have produced the indurate type of sorus if infection had occurred (table 4), in which case the spores would have been homozygous. All of the indurate sori were black, in contrast to the dark brown of the powdery sori. This color contrast probably is due to the slightly darker spores in conjunction with color intensification caused by a more effective elimination of air spaces in the indurate sorus.

It is apparent from these studies that the factor for powdery sorus type is dominant over the factor for indurate sorus type and that the segregation and recombination of factors in the F_2 occurs in a 1 : 2 : 1 ratio. Chlamydospore markings and color are known to be inherited in a similar manner (*6*).

SPORIDIAL FUSIONS

In a previous report (*4*) it was shown that in the fusion process between any two sporidia of *Ustilago avenae* and *U. levis* one sporidium is active while the other appears to be passive. Since the sporidia normally are morphologically indistinguishable it was not determined whether the active sporidia were of the same sex and the passive sporidia of the opposite sex. Recently, however, it was found possible to modify the morphology of sporidia to such an extent that those of one line can readily be distinguished from those of another line. As shown in figure 3, sporidia that bud on potato-dextrose agar are larger and more uniform in size and shape (fig. 3, *A*, *D*) than those

that bud on plain agar (fig. 3, *B*, *E*). Furthermore, sporidia from plain agar usually have no food vacuoles, while those from potato-dextrose agar may have one to several, the usual number being two.

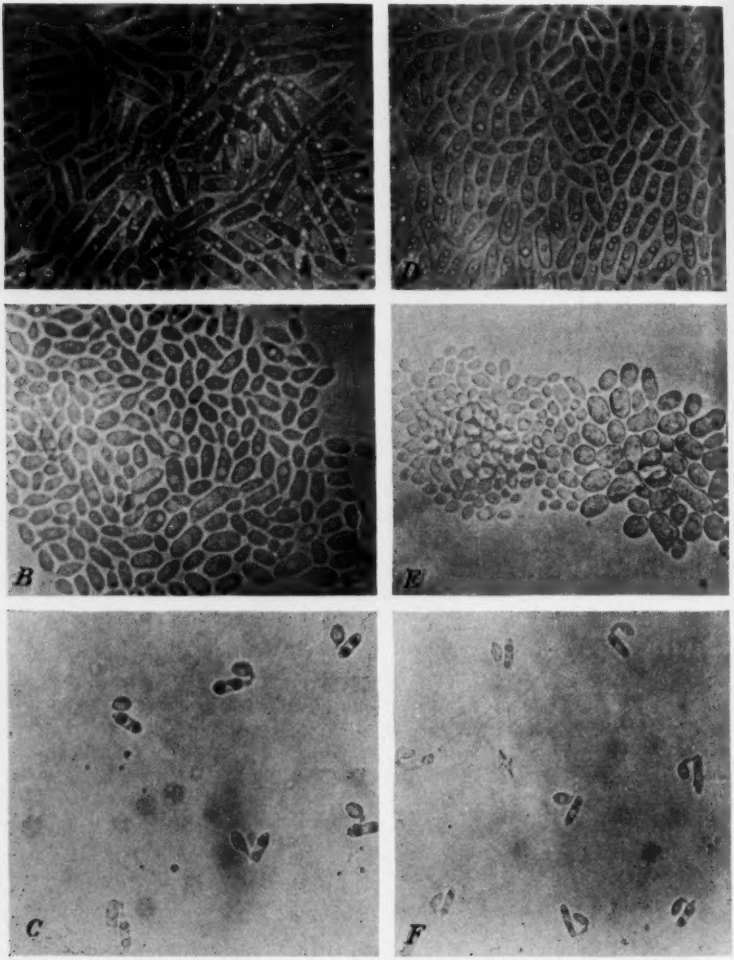

FIGURE 3.—Photomicrograph of *Ustilago levis* and buff smut sporidia showing differences in size and shape on potato-dextrose agar and plain agar and pairs of sporidia in various stages of fusion. *A*, *U. levis* 91–1 on potato-dextrose agar; *B*, on plain agar; *C*, pairs of *U. levis* sporidia from the two media, showing stages of the fusion process; *D*, buff 55–4 on potato-dextrose agar; *E*, on plain agar; *F*, pairs of the buff smut sporidia from the two media showing stages of the fusion process.

Thus, for example, by pairing the sporidia of sex A grown on potato-dextrose agar with the sporidia of sex B grown on plain agar, or the sporidia of sex A grown on plain agar with the sporidia of sex B grown

on potato-dextrose agar, it is possible to determine which sporidia are active and which are passive in the fusion process.

Such pairings, which may be made with the aid of a micromanipulator, are shown in figure 3, *C, F,* and the ease with which the sporidia are distinguished from each other is obvious. By this means observations were made on pairs of plus (+) and minus (−) sporidia of *Ustilago avenae, U. levis,* and the buff smut. In 49 pairs of *U. avenae* sporidia, the plus (+) sporidia, taken from plain agar, were active and the minus (−) sporidia, taken from potato-dextrose agar, were passive, while in 39 other pairs the minus (−) sporidia, taken from plain agar, were active and the plus (+) spordia, taken from potato-dextrose agar, were passive. In 10 pairs, however, the plus (+) sporidia, taken from potato-dextrose agar or plain agar, were active while the minus (−) sporidia, taken from either medium, were passive. Thus, in 88 pairs the sporidia taken from plain agar were active and the sporidia taken from potato-dextrose agar were passive, regardless of sex, while in 10 pairs the plus (+) sporidia were active and the minus (−) sporidia were passive, regardless of the medium from which they were taken. In 93 pairs of *U. levis* sporidia and 80 pairs of buff smut sporidia the sporidia taken from plain agar were active and those taken from potato-dextrose agar were passive, regardless of sex. Therefore, it appears from these observations that apparent active or passive participation in the fusion process is governed primarily by the "physical" condition of the sporidia and not by their sex. Obviously, those sporidia which are grown on plain agar are in a "starved" condition and apparently respond more rapidly to the fusion stimulus than those which are grown on potato-dextrose agar and have an abundance of reserve nutrients. The 10 pairs of *U. avenae* sporidia mentioned above were an exception to the general rule.

The increased tendency for sporidia of the smut fungi to fuse when placed under conditions of low nutrients is a generally recognized fact. Consequently, the mating of sporidia from a low-nutrient medium with sporidia from a high-nutrient medium might be considered an unfair test of their active and passive reactions. For this reason it seemed desirable to mate sporidia of opposite sex that had been grown on the same medium. Accordingly, observations were made on pairs of sporidia in which the larger sporidium of each pair represented one sex and the smaller sporidium the other sex, both sporidia being taken from potato-dextrose agar. Twenty pairs of buff smut sporidia were observed and the plus (+) sporidium was active in 9 pairs, the minus(−) sporidium was active in 9 pairs, and both sporidia were active in 2 pairs. Similar results were obtained with 20 pairs of *Ustilago avenae* sporidia, but no observations were made on *U. levis.* These results further indicate that sporidia of opposite sex in the oat smut fungi may appear active or passive in the fusion process, depending, at least in part, upon their "physical" condition.

In the course of the studies on sporidial fusions, two sizes of sporidia were observed in one monosporidial line of the buff smut on plain agar. This size difference can be seen in figure 3, *E,* where a colony of the larger sporidia is shown adjacent to and coalesced with a colony of the smaller sporidia. These two colonies developed from single

sporidia, and since both sizes of sporidia were present in a monosporidial line it is possible that one type arose from the other through mutation.

SUMMARY

Seven physiologic races of the buff smut of oats are described, which either arose through mutation or were produced by hybridization with *Ustilago avenae* or *U. levis*.

Mature chlamydospores of the buff smut contain a single, diploid nucleus. Reduction division accompanies spore germination and each cell of the promycelium contains a single haploid nucleus, as do the sporidia which bud from the promycelium. When two sporidia fuse, the nucleus from one passes into the other and both nuclei pass into the infection hypha. The nuclear behavior of the fungus in the host has not been studied, but presumably the dikaryophase persists throughout the parasitic stage and the nuclei fuse at the time of chlamydospore formation.

The factor for powdery sorus type is dominant over the factor for indurate sorus type. Segregation and recombination of factors for these characters occurs on a simple 3:1 ratio basis.

Active and passive participation of sporidia of the oat smuts in the fusion process appears to be dependent, at least in part, upon the "physical" condition of the sporidia rather than upon their sex. For example, sporidia that bud on plain agar respond more rapidly to the fusion stimulus than sporidia that bud on potato-dextrose agar.

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VIRUS DISTRIBUTION IN MOSAIC-RESISTANT TOBACCO AND ITS RELATION TO PATTERN DEVELOPMENT IN SUSCEPTIBLE VARIETIES[1]

By W. D. VALLEAU, *plant pathologist*, and STEPHEN DIACHUN, *assistant in plant pathology, Kentucky Agricultural Experiment Station*

INTRODUCTION

A review of the literature on virus movement in plants indicates that in mosaic-susceptible tobacco and tomato the series of events following inoculation is about as follows: Multiplication in the leaf at the point of inoculation for about 40 hours or more and then release of the virus into some part of the vascular system, where it is carried as particles for long distances without further multiplication and deposited in widely separated places in young, rapidly expanding leaves. At these points multiplication again takes place to be followed by a slow, gradual cell-to-cell movement of the virus in the immediate vicinity of the point where particles are deposited. These events are intimately concerned with pattern production in the young leaves.

The statement is often made in the literature that tobacco mosaic causes no symptoms in leaves which are not rapidly growing at the time of inoculation. If this were true one would have no way of knowing, except by detailed study, the distribution of virus in the leaves of tobacco plants, especially in the case of slowly invaded, mosaic-resistant plants. There are many field strains of the tobacco mosaic virus several of which bleach leaf tissue, around the points of inoculation, from green to bright yellow. It was reasoned that if old leaf tissue bleached when inoculated, the distribution of the virus in the leaves of a tobacco plant should make itself evident by the presence of chlorotic patterns.

If virus distribution and chlorotic patterns in old as well as in young leaves are found to correspond closely, then the plant breeder will have a valuable tool in determining the exact degree of resistance of his resistant selections, as it will only be necessary to look at an infected plant to know the distribution of virus within it, and the student of virus movement and distribution will have his problem simplified.

Ambalema, a mosaic-resistant variety of *Nicotiana tabacum* discovered by Nolla (*5*),[2] and its hybrids, not only give an opportunity to produce resistant varieties of tobacco but also provide excellent material for the study of virus movement in tobacco. Ambalema contains strains which are highly resistant to the tobacco mosaic virus, others which are invaded slowly, and still others in which fairly typical mild patterns develop. It is probable, therefore, that different investigators will report different degrees of resistance of Am-

[1] Received for publication April 15, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 247.

balema to the tobacco mosaic virus. The strain of Ambalema selected by the senior writer and used in extensive breeding studies appears to be one which is practically immune. When it is inoculated in the field with a nonnecrotic spotting (7) white mosaic virus, the local lesions develop prominently in the course of a few days as bright yellow spots which attain a diameter of 5 mm. or less. The virus appears to be completely localized in these, for no further chlorotic spots develop on the plants. That there is no barrier to rapid long-distance movement of virus in highly mosaic-resistant plants was proved by Valleau (7) when he demonstrated long-distance spread of the necrotic-spotting white mosaic virus in Ambalema which is practically immune to it. The virus multiplied in a Turkish tobacco scion grafted to Ambalema, flowed from the scion to developing leaves of Ambalema where it caused numerous scattered necrotic spots (from which the virus was recovered), and caused no further symptoms.

Hybrids of burley tobacco with Ambalema produce plants in the F_2 generation with all degrees of resistance between that of Ambalema and susceptible burley. The resistant plants in the F_2 generation are those in which the yellow or white strains of the virus appear to be completely localized in yellow spots; those in which there is delayed movement of a small amount of virus from local lesions to leaves higher on the plant, where it multiplies very slowly and causes yellow spots; those in which larger quantities of virus move into developing leaves, multiply moderately rapidly to produce ring patterns (pl. 2) but do not invade the growing point; and possibly plants of a lesser degree of resistance which are difficult to distinguish from susceptible plants except that the young leaves are mottled but not distorted. In the present study numerous mosaic-resistant burley and dark tobacco plants have been under observation both in the greenhouse and in the field during several years, but the exact distribution of virus was studied in hybrid burley plants in which local yellow spots developed when inoculated with "pure white" mosaic, and distinct yellow spots developed at points to which virus was carried higher on the plant. It is the object of this paper to report these findings and to discuss their application to pattern development in susceptible plants.

DISTRIBUTION OF VIRUS IN MOSAIC-RESISTANT PLANTS

The exact distribution of the "pure white" mosaic virus in a resistant burley plant was studied in an F_2 (F_2 burley $\times$ Ambalema) $\times$ burley. A lower leaf (leaf 2, fig. 1) was inoculated heavily. The initial spots finally coalesced to form a large yellow area. Virus was carried from this area to leaves higher on the plant where the individual particles or aggregates were distributed, multiplied, and again caused yellow spots. Invasion was largely confined to one sector of the plant as leaves on the opposite side remained free or nearly free from yellow spots.

The extent of invasion of the plant as indicated by yellow patterns is represented in black in figure 1. From the points marked *g* green disks were cut, and from the points marked *y* yellow disks were cut with a cork borer 0.4 cm. in diameter. Six disks were cut from each

leaf. The borer was sterilized in boiling water before each cut. Each disk was ground separately in 2 drops of $\frac{M}{10}$ disodium phosphate and rubbed on a portion of a leaf of a burley test plant containing the necrotic spotting factor from *Nicotiana glutinosa*. The necrotic spots

FIGURE 1.—Distribution of chlorotic patterns (black) in all the leaves of a burley plant, containing the mosaic-resistant factors from Ambalema, following inoculation with white mosaic on leaf 2. *g* indicates points from which green disks of tissue were cut and tested on a necrotic-spotting burley plant; all green disks proved to be virus-free. *y* indicates points from which yellow disks were cut all of which contained virus.

were counted 8 days later. The results of the inoculations are shown in table 1.

Disks from leaves without symptoms caused no necrotic lesions in the test plants. Green disks from all patterned leaves proved to be free from virus in spite of extensive invasion of some of these leaves and the close proximity of the green tissue to chlorotic viruliferous tissue. (See leaf 10, fig. 1.) All yellow disks proved to be from invaded areas as expected and contained a fairly high concentration of virus. Localization of virus in the yellow areas is clearly brought out. The results of this study demonstrate that in both old and young leaves of plants inoculated with a bleaching strain of the tobacco

mosaic virus, the virus causes yellow spots wherever it multiplies in leaf tissue and its distribution within the leaf is quite accurately indicated by the distribution of chlorotic spots.

TABLE 1.—*Virus concentration in yellow or white areas in a resistant burley plant as shown by the number of necrotic spots produced when macerated tissue from these areas was rubbed onto leaves of burley plants containing the N factor from Nicotiana glutinosa*

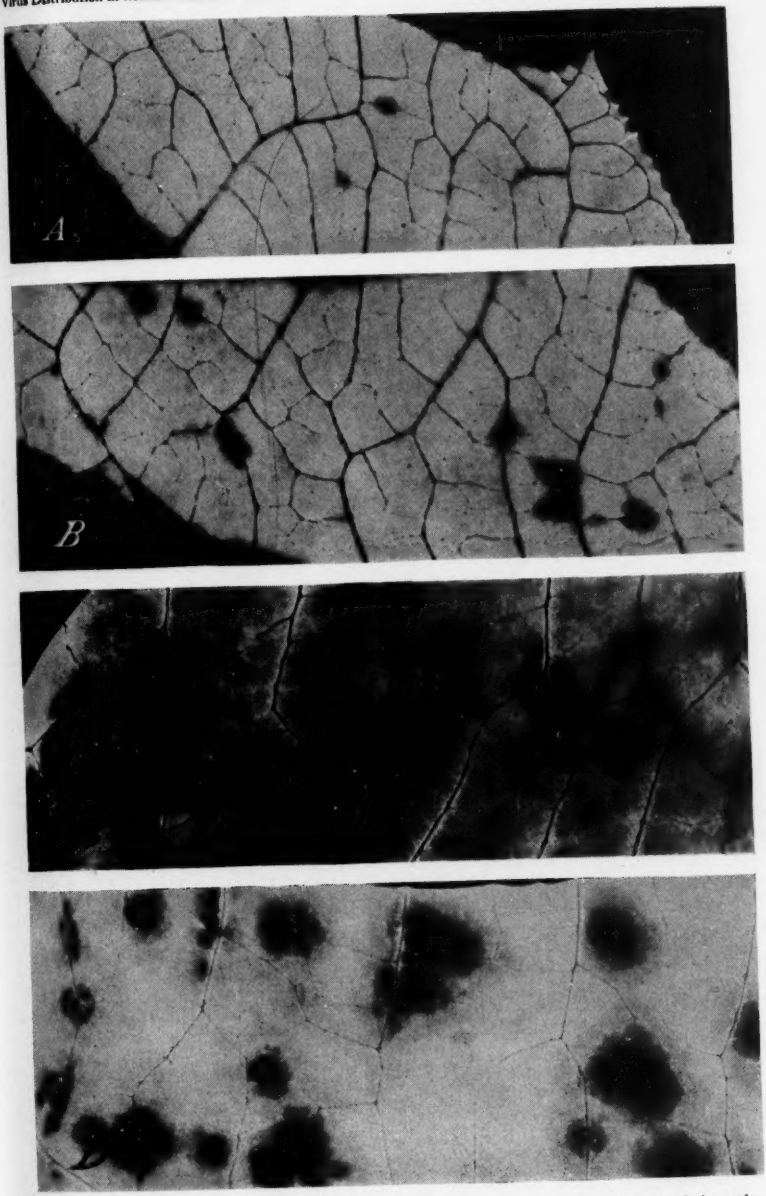
[Leaves 1, 3, 4, 6, 9, 14, 17, 19, 20, and 21 were symptomless]

Leaf No. (numbered from bottom)	Number of necrotic spots[1] produced by disks from yellow or white areas (*y*)			Leaf No. (numbered from bottom	Number of necrotic spots[1] produced by disks from yellow or white areas (*y*)		
	Disk No. 4	Disk No. 5	Disk No. 6		Disk No. 4	Disk No. 5	Disk No. 6
1				10	8	29	9
2	22	15	14	11	13	9	5
3				12	18	6	7
4				13	1	9	2
5	14	24	19	14			
6				15	10	4	4
7	18	24	10	16	15	11	9
8	26	14	7	17			
9				18	23	9	6

[1] Disks from leaves without symptoms and from green areas of affected leaves produced no necrotic spots on the test plants.

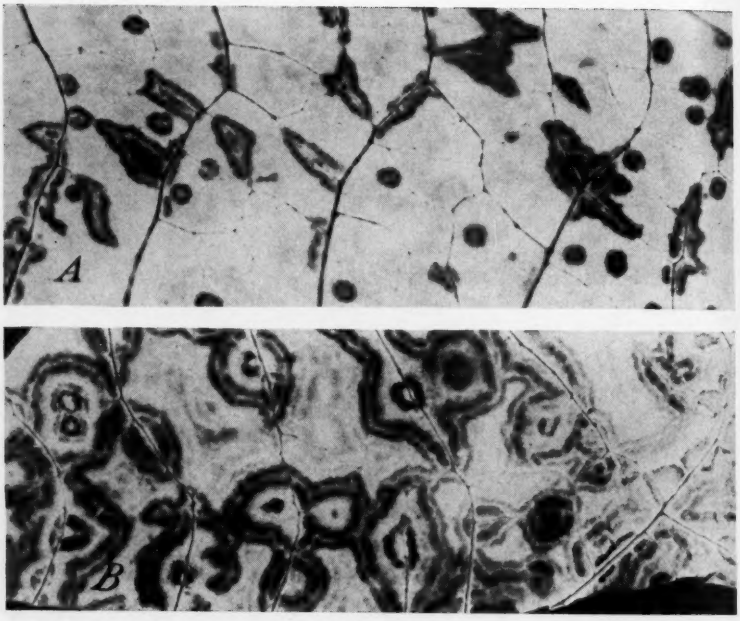
Another degree of resistance of a burley plant carrying two factor pairs for resistance from Ambalema is illustrated in plate 1. The photographs reproduced were contact prints made from leaves at various distances from the growing point of a mosaic-resistant burley plant. They are reproduced in natural size; *A*, leaf 32 is near the top of a large plant just developing a blossom bud. *B* is leaf 30; *C*, leaf 29, and *D*, leaf 24. The portion of leaf photographed is that between two secondary veins.

In leaf 32 (*A*) a few single virus particles or aggregates have been carried to points on small veins where they have multiplied, spread from cell to cell, and produced chlorotic (black on the contact print) spots. In leaf 30 (*B*) the spots are larger and somewhat more numerous. In the older leaf 29 (*C*) the spots have increased in size and are beginning to coalesce, which indicates that the tissues are slowly becoming solidly invaded. In leaf 24 (*D*) definite ring patterns are evident, and certain portions of the leaf are completely invaded. In plate 2, *A* and *B* are from another plant of the same degree of resistance; *A* represents a recently invaded leaf showing numerous centers from which the virus is slowly spreading by cell-to-cell movement; and *B* represents a leaf which has been slowly but completely invaded. The ring-spot symptom in this and other virus diseases is an indication that invasion occurred when the leaves were well developed. From the practical standpoint the degree of resistance shown in figure 1 represents immunity in the field, while the degree of resistance shown in plates 1 and 2 is such that with moderately heavy infection at setting time with a rapidly multiplying bleaching strain of virus the plants will become slowly invaded and infection will consist in scattered chlorotic ring patterns but with no distortion and only a slight retardation of growth. Inoculation after the plants have commenced rapid growth usually causes only local infections. Under farm conditions where a susceptible variety becomes heavily infected plants of the degree of resistance illustrated in plates 1 and 2 show no evidence of infection.



Invasion by white mosaic virus of a White Burley tobacco plant containing the two factor pairs from Ambalema: *A*, Leaf 32; *B*, leaf 30; *C*, leaf 29; *D*, leaf 24. Leaf 32 is from near the top of the plant; the others are numbered in descending order on the stalk. Dark areas represent chlorotic spots where virus particles have been carried through the vascular system, become lodged, and have multiplied. Contact prints made by using a portion of the leaf between two secondary veins as the "negative." Natural size.

Virus Distribution in Mosaic-Resistant Tobacco PLATE 2



Invasion by white mosaic virus of a White Burley tobacco plant of the same degree of resistance as the plant shown in plate 1: *A*, Many of the centers of multiplication of the virus are indicated by the black (white or light yellow) spots and circles, the tissue having been almost completely invaded but not yet bleached; *B*, complete invasion of the leaf tissue by cell-to-cell spread of the virus from numerous centers, the ring patterns having developed after the leaf was fully grown. Contact prints shown. Natural size.

VIRUS DISTRIBUTION AS RELATED TO PATTERN DEVELOPMENT IN SUSCEPTIBLE TOBACCO

In addition to the practical value of the use of white mosaic virus in breeding for resistance the results are of theoretical value in giving a better understanding of pattern development in susceptible plants. The tobacco mosaic virus produces several distinct sets of symptoms in tobacco plants, depending upon the strain of the virus used and the genotype inoculated. Routine breeding studies for mosaic resistance and the results of the present studies have thrown much light on the nature of these patterns, a discussion of which may tend to give those unfamiliar with mosaics a better understanding of pattern development.

The development of the ordinary green patterns produced by most of the common strains of the tobacco mosaic virus can be understood if one realizes what occurs in a slowly invaded mosaic-resistant plant. The leaves illustrated in plate 1 were well developed before virus particles invaded them. The particles were scattered through the leaves and each formed the center of a chlorotic pattern. If the growing point of this resistant plant together with the virus present were telescoped to form the growing point of a susceptible diseased plant then the development of patterns in the susceptible variety would be readily understood. The youngest leaf primordia would be virus-free, formed leaves in the bud would contain an occasional virus particle, slightly older leaves would have chlorotic areas or patterns where particles had multiplied from a few centers, and still older leaves would be patterned but would be invaded more or less solidly because of cell-to-cell invasion into healthy tissue surrounding the points of virus multiplication. A limited study of virus distribution in growing-point leaves has indicated that invasion of growing points of susceptible plants takes place in the manner described and that much of the tissue of the growing point is virus-free.

Certain of the ordinary green strains of tobacco mosaic virus cause the inoculated area on a fully grown leaf to slowly become chlorotic and then necrotic. The virus carried to leaves too old to develop patterns but young enough to be expanding rapidly, quickly invades them from numerous centers where particles are deposited, and finally causes extensive necrosis. These are the burning strains. In the younger leaves ordinary mottle patterns with but little necrosis develop.

Ring patterns are a common symptom of virus diseases (*3*). Occasionally a plant is found affected by a strain of tobacco mosaic which causes ring patterns and which produces them when transferred to other tobacco plants and to tomatoes (*2*). Plates 1 and 2 show that ring patterns develop when virus particles are distributed in fairly well-developed leaves too late to prevent the initial normal development of chloroplasts. The bleaching strains, as they advance from these centers into moderately resistant tissue, develop the "Liesegang" type of pattern made up of bands of green and yellow tissue (pl. 2.) The leaf finally bleaches to a light green or yellow. Any yellowing strain which multiplies at only a moderate rate could cause ring patterns in a susceptible variety because chloroplast development would proceed faster than virus multiplication and spread in young leaves, and the leaves would attain considerable size with no distor-

tion before the infected spots became apparent. As the virus invaded the older and more resistant tissue, ring patterns would result as in the mosaic-resistant leaves pictured in plate 2.

The patterns shown in figure 1 are typical of speckled mosaic, a disease which is of rather rare occurrence in nature (*4*). A slowly multiplying bleaching virus, rather than a slow-moving one, as postulated by Norval (*6*), can produce exactly the same effect in a susceptible plant as a rapidly multiplying one in a highly resistant plant. In each case the virus remains in the inoculated area an abnormally long time, and when released is carried to leaves higher on the plant where it again multiplies so slowly that only yellow specks develop as the leaves expand. Thus the growing-point tissue and young leaves in the bud are practically virus-free and distortion does not occur.

Masked strains such as Holmes (*1*) obtained by heat treatment have been collected by the senior writer on several occasions from solanaceous weeds collected at random in an old tobacco-growing area. On inoculated Turkish tobacco plants an occasional, faint, green ring pattern can be seen which may disappear in the course of a day or so. A masked strain seems to be in the nature of a non-bleaching, slow-multiplying strain. Young leaves are uninvaded or slowly invaded and visible patterns are not formed except for occasional faint rings.

SUMMARY

The progress of a bleaching strain of the tobacco mosaic virus can be followed accurately in a mosaic-resistant plant by the development of chlorotic patterns in invaded leaves. The virus appears to be confined almost completely to chlorotic areas, especially in old leaves. The distribution of chlorotic spots in a highly resistant plant inoculated with a bleaching strain gives further proof that virus distribution in a plant is by long-distance movement of virus particles or aggregates of particles, from points of inoculation and multiplication, followed by slow cell-to-cell movement of virus in the immediate vicinity of the point of lodgment of the virus particle. Resistance appears to result in slow multiplication of virus at the points of inoculation and slow release or complete failure of release of the virus into vascular tissue for long-distance spread. The mechanism which controls release of virus from local infections in susceptible and resistant varieties is not known.

Rate of multiplication of virus, rather than rate of actual movement of virus particles within the plant, seems to explain the difference between so-called slow-moving and rapidly moving virus strains. There appears to be no evidence that one strain moves more rapidly than another once it is released from local infections for long-distance transfer.

A study of virus distribution in mosaic-resistant plants aids in understanding pattern development in susceptible plants. Many of the different symptom complexes which occur in susceptible tobacco, such as mottled mosaic, ring mosaic, speckled mosaic, etc., which are correctly attributed to different strains of the virus, can be duplicated in resistant tobacco by the use of a single virus strain on plants of different degrees of resistance.

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VIRUS DISTRIBUTION IN THE LEAVES OF MOSAIC-SUSCEPTIBLE TOBACCO PLANTS INOCULATED AT TOPPING TIME [1]

By W. D. VALLEAU, *plant pathologist*, and STEPHEN DIACHUN, *assistant in plant pathology, Kentucky Agricultural Experiment Station*

INTRODUCTION

The extent to which tobacco plants inoculated with mosaic virus at topping time may become invaded by harvesttime is not known. The movement of virus into well-developed uninoculated leaves of tomatoes and Turkish tobacco has been shown to be slow, 3 weeks being required for the invasion of rapidly growing tomato plants, with invasion still incomplete at the end of 3 months in field-grown fruiting tomato plants.[2]

This paper presents evidence on the distribution of virus in old leaves of susceptible plants of burley tobacco (*Nicotiana tabacum* L.) inoculated at topping time and its relation to visible symptoms when a bleaching strain of the virus is used.

VIRUS DISTRIBUTION IN MATURING SUSCEPTIBLE WHITE BURLEY PLANTS

Three plants of Kentucky No. 16 burley growing in a ground bench in the greenhouse were topped and inoculated with the "pure white" mosaic virus, a strain isolated from white mosaic collected from pepper.[3] On plant 1, four immature top leaves were inoculated; on plant 2, three fully grown lower leaves were inoculated at three points equally distributed along the midveins; and on plant 3, all the leaves were inoculated at three points on one half of each leaf.

UNINOCULATED LEAVES VIRUS-FREE 31 DAYS AFTER INOCULATION OF PLANT

After the inoculation of the plants the new growth developed white mosaic. The inoculated areas became yellow and gradually increased in size. The uninoculated leaves of plants 1 and 2 remained symptomless. Thirty-one days after inoculation, six cork-borer sections 1.5 cm. in diameter were cut from each uninoculated leaf of plants 1 and 2, from green portions of inoculated leaves of plants 2 and 3, and from infected suckers of all three plants. The six disks from a leaf were crushed together in 0.5 cc. of $\frac{M}{10}$ disodium phosphate and rubbed on one-fourth of a leaf (25 to 30 cm. long) of a burley test plant carrying the necrotic spotting factor from *Nicotiana glutinosa*. The uninoculated leaves were all symptomless and proved to be virus-free (table 1). Apparently there had been no invasion of these leaves

[1] Received for publication April 15, 1940.

[2] BAWDEN, F. C. PLANT VIRUSES AND VIRUS DISEASES. 272 pp., illus. Leiden. 1939. See ch. 13.

[3] See JOHNSON, E. M. VIRUS DISEASES OF TOBACCO IN KENTUCKY. Ky. Agr. Expt. Sta. Bul. 306, pp. 385–415, illus. 1930.

TABLE 1.—*Number of necrotic spots produced on burley test plants when inoculated with disks cut from 2 Kentucky No. 16 burley tobacco plants 31 days after inoculation on top 4 and basal 3 leaves, respectively*

Plant No.	Part sampled	Number of disks	Necrotic spots produced
1	Leaves 1 to 11	66	0
	Leaf 12 (inoculated)	6	500
	Top suckers	6	400
	Basal suckers	6	500
2	Leaves 4 to 14	66	0
	Leaves 1 to 3 (inoculated, green areas)	18	0
	Basal suckers	6	400
	Top suckers	6	400

in the 31 days following inoculation. The inoculated leaves on plant 1 (upper) were spotted with numerous yellow spots and contained a high virus concentration. They appeared to be almost completely invaded. The mottled suckers, as would be expected, contained a high virus concentration. The uninoculated leaves of plant 2 and the green portions of the inoculated leaves (mature when inoculated) were symptomless and virus-free. The results indicate a rapid development of mosaic virus in young tissue (upper inoculated leaves of plant 1 and suckers) and an extremely slow cell-to-cell invasion of older leaf tissue (inoculated leaves of plant 2).

VIRUS INVASION OF UNINOCULATED LEAVES

Forty days after inoculation some of the middle leaves of plant 1 and the two upper leaves of plant 2 showed some chlorosis of the leaf-blade tissue along the bases of the midveins. After 45 days five leaves of plant 1 and the two upper leaves of plant 2 were chlorotic along the midvein and the secondary veins. After 52 days one more leaf on plant was becoming chlorotic and after 59 days all but two leaves of plant 2 showed chlorosis along the base of the midrib (fig. 1). The invasion of these leaves was slow and evidently from cell to cell following the larger veins and slowly spreading from them into the blade tissue. From these results it is evident that the white mosaic virus can produce prominent symptoms in the invaded portion of old mature leaves.

VIRUS DISTRIBUTION IN INOCULATED LEAVES

After 31 days the inoculated leaves of plant 1 (four top leaves) were extensively invaded, as has been shown. The inoculated leaves of plant 2 (three bottom leaves) were mature at the time of inoculation and showed comparatively little invasion as indicated by chlorotic patterns. The leaves of plant 3 were in all stages of development from mature basal leaves to recently expanded top leaves when inoculated. Individual disks were cut from isolated green areas, from green areas near yellow areas, and from yellow areas of the inoculated leaves of plants 2 and 3. The disks were tested on burley test plants. The distribution of yellow patterns on the inoculated leaves, the location of the disks selected, and the results of inoculations with the disks are shown in figure 2, *A*, for plant 2 (31 days after inoculation) and in figure 2, *B*, for plant 3 (37 days after inoculation).

Figure 2, *A* and *B*, shows the slow development of chlorotic yellow patterns in old leaves inoculated more than 30 days previously, and

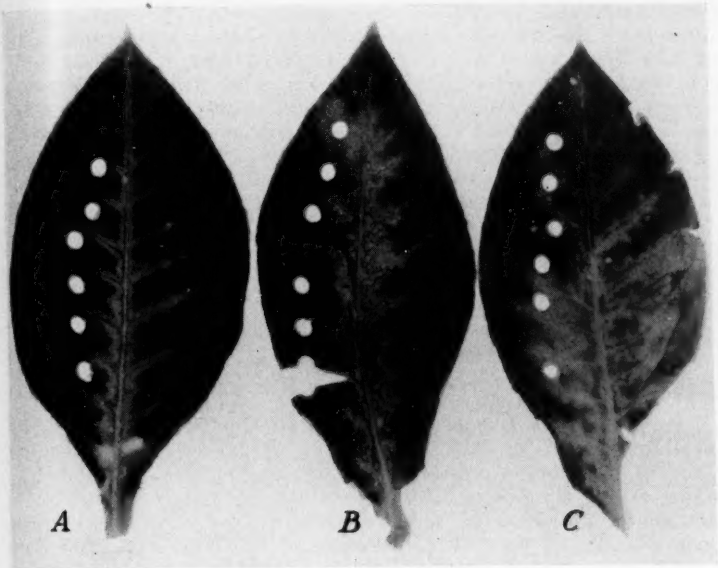

FIGURE 1.—Spread of pure white mosaic virus into old leaves of topped Kentucky No. 16 burley tobacco plant 1, which was inoculated at the top February 4, 1939. These leaves were symptomless until March 6, 1939. Photographed April 6, 1939. *A*, Eighth, *B*, sixth, and *C*, fifth leaf from bottom. Samples taken 31 days after inoculation were virus-free.

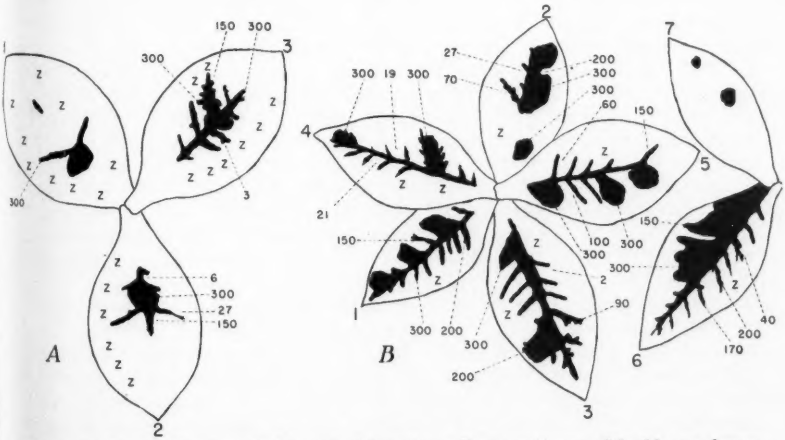

FIGURE 2.—*A*, Representation of chlorotic yellow patterns (black) on lower inoculated leaves 1, 2, and 3 of plant 2, 31 days after inoculation; *z* indicates points from which cork-borer sections were taken which produced no necrotic spots on test plants. The figures indicate the number of necrotic spots on test plants inoculated with sections taken at the end of the dotted lines. *B*, the same as *A* for plant 3, 37 days after inoculation. Leaves are numbered from below upward.

demonstrates that virus distribution is fairly accurately indicated by the yellow patterns in a susceptible plant. Yellow areas contained a high virus concentration, green areas located very near yellow areas were virus-free or contained a low virus concentration, while well-isolated green areas were virus-free. On plant 3 (fig. 2, *B*) when inoculations were made at three places on one side of the midvein the virus gradually spread to the midvein, crossed it, and spread slowly out along the secondary veins. Except for initial long-distance carriage of virus following inoculation there is no evidence of any type of spread in these leaves other than slow cell-to-cell movement. The fact that the patterns follow the veins probably has nothing to do with veins as conductors of virus, but means only that there is an abundance of parenchyma tissue in which the virus can multiply. The value of a white strain of the virus in a study of virus distribution is evident.

COMPARISON OF FIVE VIRUS STRAINS

The study of virus spread in susceptible burley plants was continued by the use of nine plants for inoculations with five distinct strains of the tobacco mosaic virus. One strain was the pure white mosaic used in the previous test, two were collected as white mosaics but were recognized as different strains, one was a yellow mosaic, and one a "burning" strain of a green mosaic. The plants inoculated were grown in a greenhouse ground bench and were as large as vigorously growing field plants. Each was topped at a height of about 5 feet. Plants were inoculated on the tips of the top leaves (plants 1, 3, 5, and 7) or on the edge of one side of lower leaves 6, 7, and 8 about midway between tip and base (plants 2, 4, 6, and 8) or on the top three and lower three leaves (plant 9). Records were kept of the time of invasion of the various suckers, appearance of local lesions, visible spread into veins of inoculated leaves, and spread into the base of uninoculated leaves. The records are given in table 2. Symptoms appeared about as follows: Chlorosis in inoculated areas in about 9 days, with vein clearing in leaves of the top suckers appearing almost simultaneously on top-leaf-inoculated plants, and about 6 days later in bottom-leaf-inoculated plants. Vein clearing in the lower sucker appeared 5 to 11 days after the local lesions, and in all instances but two occurred after the first appearance in the top suckers. This may have been caused in part by the slower growth of the bottom suckers. Visible spread of symptoms along the veins of inoculated leaves required from 16 to 29 days, during which time the chlorotic area at the point of inoculation gradually increased in size. Uninoculated leaves were uninvaded so far as visible symptoms were concerned until 30 days after inoculation, when one leaf showed infection. By the 38th day after inoculation other leaves had developed chlorosis. After that invasion was gradual along the midvein and laterals. The blade tissue was the last to be invaded. The burning strain of the virus on plant 9 caused necrosis around the inoculated areas, and 55 days after inoculation the midveins of all leaves showed chlorosis caused by slow spread of the virus. As the burning strains of the virus are commonly found in the field this observation proves that at least some of the common field strains cause symptoms in old as well as in young leaves.

TABLE 2.—*Time of appearance of symptoms in various parts of tobacco plants inoculated with five strains of the tobacco mosaic virus*

Plant No.	Virus	Leaves inoculated	Number of days required for first appearance of—				
			Local lesions	Vein chlorosis in top suckers	Vein chlorosis in lower suckers	Chlorosis in some veins of the inoculated leaf	Chlorosis of midveins of uninoculated leaves
1	White A	Top 3	10	9	16	19	38
2	do	Bottom 6, 7, and 8	9 to 16	16	16	----	----
3	White B	Top 3	9	9	16	19	38
4	do	Bottom 6, 7, and 8	10	16	21	29	43
5	Yellow	Top 3	9	10	16	16	30
6	do	Bottom 6, 7, and 8	9	16	17	24	43
7	Pure white	Top 3	9	9	14	16	38
8	do	Bottom 6, 7, and 8	9	17	14	19	47
9	Burn mosaic	Top 3 and bottom 6, 7, and 8.	24	9	16	31	43

Twenty-eight days after inoculation 6 disks 1.5 cm. in diameter were cut from the right side of each uninoculated leaf with a cork borer. The 6 disks were ground together in 0.5 cc. of $\frac{M}{10}$ disodium phosphate and rubbed on an interveinal area of at least 40 cm.^{2} of a burley test plant. From the inoculated leaves 12 disks were cut, 6 from green and 6 from chlorotic areas. The results of the inoculations are given in table 3. They indicate that 28 days after inoculation there had been practically no invasion of uninoculated leaves by any one of the five virus strains used; that invasion of inoculated leaves was not yet complete, with the possible exception of the top leaves inoculated with the burning strain; and that the distribution of the virus is indicated fairly accurately by the development of chlorosis as healthy tissue is invaded. There appears to be no significant difference in virus distribution within the plant between any of the five virus strains tested, which suggests that results obtained with the strain pure white can be considered as about typical of what may be expected from the ordinary field strains so far as plant invasion is concerned.

TABLE 3.—*Approximate number of necrotic spots produced on 40 cm.^{2} of leaf of burley test plants rubbed with a composite of six disks per leaf*

[*y* indicates disks from chlorotic areas, all others from green areas; disks cut 28 days after inoculation]

Leaf No. (numbered from the bottom)	Number of necrotic spots produced on plant No.—								
	1	2	3	4	5	6	7	8	9
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0 *y*-[1] 0
6	0	0 *y*-150	0	0 *y*-30	0 *y*-[2] 60	0 *y*-200	0	0 *y*-100	0 *y*-70
7	0	0 *y*-150	0	0 *y*-150	0	0 *y*-100	0	0 *y*-70	0 *y*-65
8	0	0 *y*-80	0	0 *y*-200	0	0 *y*-200	0	0 *y*-80	0
9 to 16	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0 *y*-100	0	3
18	0	0	0 *y*-150	0	0	0	2 *y*-150	0	[3] 200 *y*-200
19	0 *y*-200	0	0 *y*-150	0	0	0	0 *y*-70	0	[3] 200 *y*-200
20	50 *y*-200	0	30 *y*-200	0	0	0	----	0	[3] 200 *y*-200
21	60 *y*-200	----	----	0	0	0	----	0	----
Leaf from 1 top sucker	200	150	200	200	40	200	70	200	200

[1] Leaf old and generally chlorotic and green burning strain which does not cause much chlorosis.
[2] Leaf invaded at base of midrib.
[3] Green areas slightly mottled.

VIRUS DISTRIBUTION IN FIELD-GROWN PLANTS

A study somewhat similar to the greenhouse studies recorded above was made on field-grown Kentucky No. 16 burley plants. The presence of chlorosis was used to indicate the distribution of the white mosaic virus within the plant. One hundred and twenty White Burley plants were inoculated immediately after topping as follows: 30 plants on the tip of the upper leaf; 30 plants on the tip of a lower green leaf; 30 plants on the broken end of the stalk left when the top was broken out; and 30 plants topped with hands wet with juice from a pure white mosaic plant.

The results at the end of 25 days when the remainder of the crop was harvested can be briefly summarized. Plants inoculated on the tip of the upper leaf had developed local chlorotic lesions around the points of inoculation, and chlorosis had spread a few inches along the midvein and lateral veins or throughout the leaf, depending upon whether the leaf was fully grown or was immature at the time of inoculation. The top suckers developed mosaic. No leaves other than the inoculated leaf and sucker leaves showed any sign of invasion. Only 3 of 30 plants inoculated on a lower leaf developed mosaic. The season was dry and the lower leaves yellowed and died before harvest. Of those inoculated on the broken end of the stalk all but 1 developed mosaic in the upper suckers; 10 less mature plants showed occasional yellow spots up to 2 or 3 inches in diameter and occasional yellow veins on the upper 2 or 3 leaves. The virus had evidently been carried rather quickly into these leaves, as many of the spots were present on them within 10 days after inoculation and increased in size during the next 2 weeks. The remaining leaves on these plants were free from chlorotic spots and both they and the green portions of the invaded leaves presumably were virus-free. All of the plants inoculated by topping with contaminated hands developed mosaic in the upper suckers and 16 plants had scattered chlorotic veins on the upper 2 or 3 leaves similar to those in the group inoculated on the broken end of the stalk. The more advanced the stage of bloom at topping time the less the likelihood of invasion of the upper leaves.

SUMMARY

From the greenhouse and field studies reported here it appears that highly susceptible burley tobacco is invaded very slowly following inoculation at topping if the leaves have attained almost full size when the plant is inoculated. The top leaves of plants not yet in bloom become invaded by the virus in scattered spots, but the leaves age sufficiently before the virus becomes general so that it is localized to some extent in spots, which slowly increase in size. The advance of virus into mature leaves is extremely slow. At the end of a month uninoculated leaves of inoculated greenhouse plants were virus-free; at the end of 2 months the midveins and laterals of some leaves were invaded, though the blade tissue still remained free from virus. From the practical standpoint the results mean that infection at topping can have little or no influence on quality of the cured leaf of burley tobacco if the plants are topped in the full-bloom stage. If they are topped early, when the flower buds are just beginning to show, some upper leaves may be affected.

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